Medical Lib.
JAN 7 1924

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

PAGE

PHYSIOLOGICAL EFFECTS OF AIR CONCUSSION. *D. R. Hooker* 219

THE GLUCOSE EQUIVALENT OF INSULIN IN DEPANCREATED DOGS. *Frank N. Allan*..... 275

STUDIES ON FACTORS PRODUCING A RAPID INCREASE OR DECREASE IN THE NUMBER OF RED AND WHITE CELLS IN THE BLOOD STREAM. I. ACIDS AND BASES. *Ernest F. Bostrom* 291

ANTINEURITIC VALUE OF HOG MUSCLE. *Ralph Hoagland*..... 300

VAGUS APNEA. *Walter J. Meek*..... 309

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS. XVI. THE NORMAL BLOOD SUGAR OF PIGEONS AND ITS RELATION TO AGE, SEX, SPECIES AND CERTAIN DISEASES. *Oscar Riddle and Hannah Elizabeth Honeywell*..... 317

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS. XVII. BLOOD SUGAR AND OVULATION UNDER INACTIVITY OR CLOSE CONFINEMENT. *Oscar Riddle and Hannah Elizabeth Honeywell*..... 333

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS. XVIII. EFFECTS OF THE ONSET OF COLD WEATHER ON BLOOD SUGAR AND OVULATION RATE IN PIGEONS. *Oscar Riddle and Hannah Elizabeth Honeywell*..... 337

THE EFFECT OF THE ORAL ADMINISTRATION OF ALCOHOL UPON THE BLOOD SUGAR OF RABBITS. *N. R. Blatherwick, L. C. Maxwell and M. Louisa Long*..... 346

STUDIES ON THE RELATIVE PHYSIOLOGICAL VALUE OF SPECTRAL LIGHTS. IV. THE VISIBILITY OF RADIANT ENERGY. *Henry Laurens*..... 348

HEAT REGULATION AND WATER EXCHANGE. I. THE EFFECTS OF HOT AND COLD BATHS UPON BLOOD CONCENTRATION AND BRAIN VOLUME IN DOGS. *Henry G. Barbour*.... 360

HEAT REGULATION AND WATER EXCHANGE. II. THE RÔLE OF THE WATER CONTENT OF THE BLOOD, AND ITS CONTROL BY THE CENTRAL NERVOUS SYSTEM. *Henry G. Barbour and Edward Tolstoi*..... 373

HEAT REGULATION AND WATER EXCHANGE. III. THE EFFECTS OF "DRY" AND "MOIST" HEAT UPON THE BODY TEMPERATURE AND BLOOD CONCENTRATION OF DOGS. *Ezra Lozinsky*..... 388

HEAT REGULATION AND WATER EXCHANGE. IV. THE INFLUENCE OF ETHER IN DOGS. *Henry G. Barbour and Wesley Bourne*..... 399

THE EFFECTS OF EXTREME TEMPERATURES ON FISHES. *S. W. Britton*..... 411

A STUDY OF VASECTOMY ON RATS AND GUINEA PIGS. *Robert Oslund*..... 422

VOL. LXVII—No. 2
Issued January 1, 1924

BALTIMORE, U. S. A.
1924

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 3, 1918

Made in United States of America

MERCURY MANOMETER WITH THREE-WAY COCK

THIS manometer is constructed of carefully selected calibrated, annealed glass tubing, which is mounted on a hard rubber block. The horse-hair and weight of the actual manometer are replaced by a slotted brass tube which serves as a guide for the writing needle, assuring continuous approximation of writing point to paper. The writing point is brought into contact with the paper by turning a screw which rotates the brass guide tube. The scale is not fixed, as in other types of manometer, but can be adjusted at "blood pressure zero" for each experiment. The three-way cock serves to put into communication the anti-clot reservoir, the manometer and the blood vessel.

PRICE $23.00

Catalogue on Request

JOSEPH BECKER

437 WEST 59TH STREET **NEW YORK**

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 67 JANUARY 1, 1924 No. 2

PHYSIOLOGICAL EFFECTS OF AIR CONCUSSION[1]

D. R. HOOKER

From the Physiological Laboratory of Johns Hopkins University, Baltimore

Received for publication September 15, 1923

The physiological effects of air concussion were studied during the period of the war because of the probable relationship of air concussion to certain war injuries. Men subjected to the concussion of large shells often developed a condition of "shock" which was unrelated to obvious traumatism since no external or internal wounds were clinically demonstrable. While it is true that subsequent investigations developed the fact that many of these instances were cases of psychoses (shell shock), nevertheless a considerable number were left, the symptomatology of which indicated some physical injury (1) without, however, any external manifestation.

The study was subsidized by a grant from the National Research Council and was developed as a component part of the investigations of the Committee on Shock of the Medical Section of the Council. The writer wishes to express his obligation to the officers of the Council, without whose aid the facilities enjoyed would have been quite unattainable.

It may be at once stated that the exposure of animals, anesthetized and unanesthetized, to a sufficient air concussion leads to primary shock, i.e., a condition of low arterial blood pressure unassociated with hemorrhage and exposure. The work was done in the winter months of 1918 and 1919 at the Sandy Hook Proving Ground, and consisted largely in studying the effects of exposure of animals close to the large-caliber guns used in powder testing. The facilities made available for the investigation by the Commanding Officer of the Proving Ground were excellent, a fact

[1] Publication of this paper has been long withheld in the hope that those who undertook to study the pathological material might be able to report conjointly. After five years the writer is convinced that this hope cannot materialize. A preliminary report was made in April, 1919. See this Journal, 1919, xlix, 121.

which speaks both for the interest of the Ordnance Department in furthering extra-departmental investigations and for the ability of the officers of the Research Council to correlate scientific work in a branch of the government which was especially harassed by the emergency of the situation.

No data are available to show the number of cases of "primary shock" resulting from warfare, nor to indicate the part played by air concussion in producing the condition. The so-called "shell shocked" cases, at first ascribed to the concussion due to the detonation of high explosive, were subsequently placed in a category of psychic upsets to which the explosion contributed only incidentally. It is, of course, conceivable that the concussion might establish a central nervous system lesion primarily associated with the psychic upset, but indirect evidence and opinion at the present time are against this view. It is likewise conceivable that concussion might contribute factors of injury which become accentuated in the condition of "secondary shock" due to wounds, exposure and loss of blood. But here again indirect evidence indicates a sufficient cause in the tangible factors as opposed to the hidden effects of concussion. There remains a relatively small group of cases of "primary shock" associated with the detonation of explosives which are undoubtedly analogous to the experimental cases considered in this paper. In addition some of the "secondary shock" cases as well as some of those diagnosed as "shell shock" may well be associated with an injury due to air concussion and would constitute borderline cases of "primary shock." Indeed, the more recent literature emphasizes the probability that too much emphasis was placed on psychoses and too little upon the, at the time, intangible physical injury to the central nervous system (2).

The present paper brings together a number of observations illustrating the sequence of events which lead to death in primary shock induced by air concussion. This sequence is of moment because since shock in these experiments is developed with remarkable suddeness, it is possible to show that some of the symptoms, i.e., acidosis, develop secondarily and cannot be regarded as of primary causative significance. Such a fact, demonstrable only in concussion shock, is undoubtedly applicable in other forms of shock.

The term "shock" has been used very loosely both in connection with injuries and ailments developing out of the late war and with similar morbid manifestations found occasionally as the result of accident in time of peace. For the purpose of this paper the definition is accepted which emphasizes the sudden development of an arterial blood pressure of approximately half normal which comes on as the result of some "shock," in this case as the result of aerial concussion. The fall in blood pressure is but one item of a symptom complex which has been extended to include

abnormal manifestations in almost every bodily process. Indeed, it is sometimes stated that shock may develop with a normal blood pressure. However, it has seemed best to specify the condition according to a single, easily demonstrable reaction.

In dealing with animals, and in the majority of cases with anesthetized animals, it follows that this report wholly excludes conditions analogous to the war psychoses described as "shell shock." Furthermore, the criterion accepted delimits these experiments to a consideration of primary shock—shock which develops suddenly upon a previously normal condition—as contrasted with secondary shock in which the characteristic symptoms develop late as the result of injury, hemorrhage and exposure. The paper is directed to a study of the physiological effects of air concussion. The data gathered may prove of some value in the interpretation of the causes of shock as seen clinically, but it should be emphasized that the use of this clinical term is simply a matter of convenience. There are today too many kinds of shock to make the term of much significance. Nevertheless it carries a meaning which could otherwise be developed only by extended phraseology, and it is this fact which has justified its use in the present content.

METHODS. Experimental observations were made upon a few cats and rabbits and upon a considerable number of frogs and dogs. As a first step in the work a study was made of the effects produced upon a few unanesthetized animals. Subsequently dogs under massive doses of morphia were used exclusively and the results obtained in this series constitute the bulk of the data reported. The preliminary experiments on frogs, rabbits, cats and dogs, without anesthesia, established the fact that the gun-blast pressure produced a condition analogous to shock. Thereafter interest centered in an analysis of the condition and for this purpose anesthesia was essential because it made control observations possible. Anesthesia was never used in the case of frogs. Except in the early experiments in which wooden cages were used, the animals were exposed in strong cloth bags which were held in position by heavy wire attached to the gun carriage.

The physiological methods employed were extremely simple and require only passing reference. Arterial blood pressure was taken with a mercury manometer, the femoral artery being used. The values given for this pressure represent, therefore, the mean arterial pressure. The venous blood pressure was obtained, as a routine in the femoral vein, using a straight tube water manometer of 3 mm. bore. A lateral opening in the tube to the cannula led to an adjustable pressure bottle. The system was filled with physiological salt solution. When a determination of the venous pressure was to be made the fluid in the manometer was raised slightly above the assumed venous pressure, the pressure bottle was

excluded by a clip and the clamp on the vein was removed. The fluid in the manometer then ran into the vein until pressure equilibrium was obtained, at which point the venous pressure reading was taken.

Respiration was recorded by graphic registration of the chest movement. This method was ill-adapted to the needs of the case because the chest-band could not be held in a fixed position during the manipulations of exposure. Consequently only the rate of respiration was obtained.

Blood samples were drawn either with a syringe from the exposed external jugular or from a cannula in the carotid artery, a small portion of blood being allowed to waste before the sample was taken. These samples were used for *a*, determinations of the catalase content of the blood according to Burge (3); *b*, attempted determinations of blood volume, and *c*, determinations of the alkaline reserve according to Van Slyke (4). I am indebted to Doctor Van Slyke for making these determinations at the Rockefeller Institute. The experimental conditions at Sandy Hook were such that work of this nature could not be carried out.

Under the heading of methods may also be considered some of the environmental factors bearing upon the problem in hand. At the time these experiments were carried out, the Sandy Hook Proving Ground was chiefly occupied in powder testing. Powder samples presented for test must first be brought to a constant temperature by storage in magazines. When the test is run it is the practice to build up the powder charge from a safe minimum to an excess charge in four rounds, then to drop back to the standard charge and to fire a series of rounds for projectile velocity determinations. If, however, several lots of powder are ready for test, they may be run together with a resultant change in the sequence of procedure. Consequently it was found exceedingly difficult to establish constant experimental conditions with respect to the air concussion administered even when a gun, which had previously given desired results, was in use. It was hoped that actual measurement of the pressure to which the animal was exposed by the Baroscope method devised by Professor Miller[2] would

[2] Prof. D. C. Miller, of the Case School of Applied Science, at the instigation of the Research Council, undertook a coöperative study of the physical factors involved in these experiments. His full report is not yet available for reference. The Baroscope mentioned in the text above measured the maximum pressure. The instrument consisted of a metal diaphragm, the displacement of which by a wave of pressure caused a needle to move. The extent of movement of this needle, previously calibrated under known atmospheric pressures, therefore, recorded the maximum atmospheric pressure to which the instrument was exposed. Some of the data for the gun-blast pressure thus obtained are made use of in the text. (The instrument is described in the Physical Review, 1919, xiv, N.S., 450.)

As will be pointed out, the maximum pressure to which an animal is exposed is not, as such, the determining factor in producing shock. The duration of the high pressure and possibly the oscillations of atmospheric pressure produced by the gun blast are physical factors in regard to which we have no information and which are very significant for the complete analysis of the problem of concussion shock.

obviate this difficulty but, as will be pointed out later, this was of very little practical help. The difficulty of correlating these physiological experiments with the procedure of powder testing to the end that approximate constant conditions should prevail, entailed much labor and waste effort. The results of French observers (5) and of Crile (6) suggested that the use of high explosive might obviate many of the difficulties encountered when depending upon the powder tests. The Chief of Ordnance generously granted the use of high explosive (trinitrotoluene) and facilities for experimentation. The practical advantages of this method are obvious in that experiments could be run independently of the routine of the Proving Ground. Unfortunately, however, it developed that the results obtained were unlike those which resulted from exposure to the gun blast and did not simulate the shock condition. The use of high explosive was therefore abandoned.

The guns are permanently mounted and usually fire at an elevation of approximately one degree. The mount carried the gun some ten feet above the ground, which slopes away in front. The blast spreads in a rapidly widening cone which is apparent in the cut made in the ground. This cut is sharply marked five or ten feet in front of the muzzle of the gun and fades rapidly away although the blast and shock may affect ground materials for a distance of fifty feet. Miller's pressure determinations indicate that the area of highest pressure lies within this cone, that the point of maximum pressure drifts backward and forward along the line of fire a distance of five, ten or even fifteen feet under presumably absolutely constant conditions, and that the pressure is dissipated with remarkable rapidity. It follows, therefore, that one cannot select a spot on the ground in front of a particular gun, firing a particular charge, and say that the pressure will be constant at different times. This was borne out in the exposure of animals; if a shock condition was produced in one exposure, another dog exposed under like circumstances might yield quite different results, that is, it might show little effect or be killed.

The study of the majority of animals bore upon the effects of a single exposure. In a few cases the animals were exposed more than once but the results thus obtained were complicated and of less interest. The physical effects produced by the firing of these large guns or by the detonation of high explosive are due to the expansion in gaseous form of the explosive substance. When a large gun is fired one experiences two distinct sensations. The first sensation is due to the "ground shock" which travels more rapidly than the sound waves and at a sufficient distance is felt an appreciable time before the sound is heard. This "ground shock" is not distressing even close to the gun; gun crews are subjected to it at frequent intervals with no ill effects, and concrete emplacements and similar structures are not injured. The "ground shock" may therefore be excluded as

a causative factor in the condition under investigation. The second sensation is due to the sound. Its intensity is determined by one's proximity to the gun. Coincident with the sound window glass may be broken and doors thrown open. The shattered glass always falls outwards and doors likewise always open outwards. It has been suggested and accepted by many that these phenomena are due to a negative pressure and that any injurious effects caused by the firing of large guns must be due to the wave of negative pressure. If windows are freely opened so that pressure conditions are readily equalized the glass is not then broken. Professor Miller has shown, however, that the largest but not the longest effect is due to the primary wave of positive pressure.

The animals were placed within the area of the gun blast and were affected, therefore, by

1. The initial air concussion as a single blow.

2. The air vibrations associated with the production of the sound. Obviously these vibrations were close to the maximum amplitude, i.e., the sound was at its lowest.

The Baroscope used by Professor Miller recorded the maximum positive pressure. In an extended study of the maximum pressure areas around different guns, Professor Miller found the highest pressure recorded by this instrument in front of the twelve-inch mortar. And yet in no case was the condition of shock produced by exposure of dogs to this gun. On the other hand, the ten-inch rifle which recorded an appreciably lower pressure on this instrument (281 pounds per square inch as opposed to 388 pounds per square inch) repeatedly produced shock. It follows in consequence of these observations that the height of the initial pressure wave as such is not the cause of shock.

Two other factors in the atmospheric disturbance require consideration. Guns of different character (mortars and rifles) and of different caliber produce different and characteristic sounds, i.e., impart to the atmosphere characteristic tonal vibrations. Along with this difference there is a difference in the length of time in which the atmosphere is disturbed. The high-pitched, short bark of the small-bore rifle is very different from the low-pitched and prolonged boom of the ten- and twelve-inch rifles, not alone in the pitch of the sound but in the length of time which the sound lasts. There is undoubtedly a similar difference in the duration of the initial pressure wave. In other words, the following atmospheric factors require attention:

1. The duration of the initial pressure wave as distinguished from its height,

2. The rate and amplitude of the vibrations imparted to the air, and

3. The duration of the vibratory disturbance.

Mairet and Durante (2), who studied particularly the pathological lesions in the central nervous system of rabbits exposed to the detonation of high explosive, emphasize the fact that the lesions which they found occur in parts especially susceptible to injury by forceful vibration, namely, the spinal and cranial nerve roots as they pass through the bony foramina. These authors take the position, therefore, that it is the *commotion* which is responsible for the injury. A similar position is taken by Carver and Dinsley (2). Mott (30), on the other hand, apparently looks upon the initial positive and negative waves of pressure as the causative factor.

Differences in the vibration rate in accordance with the ideas of these writers might explain the different results with the twelve-inch mortar and the ten-inch rifle described above, but the amplitude of such vibrations as a factor in the results is excluded by the fact that the atmospheric disturbance set up by the mortar had a higher initial propelling force than did the rifle, and this greater force must have established pressure waves of greater amplitude.

The duration of the initial pressure wave—the blow struck by the air concussion—and the duration of the resulting atmospheric vibrations, however insignificant to the unaided senses, cannot well be discarded in the absence of physical measurements. The resistance of the tissues and particularly of the bony structures would hinge in no small degree upon this factor. Dogs, shocked by air concussion, show none of the hemorrhagic lesions in the central nervous system described by Mairet and Durante in the rabbit and by Mott in man. A difference in the morbid anatomy is to be expected in different species of animals. Nevertheless if the results are to be ascribed to the vibrations alone we should expect the lesions established to be uniform in different species. Rabbits, with a weaker bony defence, are more easily injured by air concussion than are dogs. There is no anatomic basis for the belief that the atmospheric commotion would shake the nerve roots emerging from the spinal canal less in dogs than in rabbits. On the other hand, it is reasonable to assume that a given pressure would be the more effective in overcoming the resistance of the skull the longer it was maintained, and if maintained long enough it would be transmitted to the underlying tissues with the probability of doing injury to them.

By such reasoning it follows that we cannot accept the vibration factor alone as sufficient to account for the results obtained. The duration factor, then, must be considered as of prime significance.

This conclusion is borne out by Professor Miller's observations. He states (personal communication) "there is no true vibratory wave-form; there is a single positive (compression) pulse, rising rather abruptly to a maximum, and falling to a negative value (rarefaction). The numerical value of the negative pressure is usually much less than that of the

positive pressure, but it lasts for a longer time. On the return of the pressure to the normal value there is some fluctuation which shows a billowy action having a kind of irregular vibratory motion."

The observations upon which this statement is based were made with the Phonodeik, an instrument devised by Miller for the graphic analysis of sounds. The instrument is of such delicacy that it could not be used close to the gun but there is every reason to believe that the character of the sound remains unchanged regardless of the distance from its source.

Miller's physical measurements of the atmospheric disturbances set up by the gun fire therefore exclude *a*, the finer vibrations of the air which are associated with sound and *b*, the magnitude of the positive pressure in the blast area as causative factors in the production of shock. There remains then as the probable causative factor the duration of the positive pressure phase with which the results here reported are in entire agreement.

The animals were exposed to the gun blast and were therefore surrounded by the combustion products of the powder. When nitro-cellulose powder is burned the combustion products are estimated as follows:

	percent
CO_2	31.8
CO	22.7
H_2O	4.5
H_2	29.5
N_2	11.4

Mott (7) considers carbon monoxide poisoning a factor in many cases of concussion shock and particularly in cases exposed to concussion in trenches and dug-outs where the gases from high explosives would be only slowly dissipated in the surrounding atmosphere. In the present experiments the animals were exposed in bags on the flat ground and there was usually a fair wind blowing so that the gaseous products of combustion must have been very rapidly dissipated. Also the blast of the gun accelerated a mixture of the smoke and gases with the atmospheric air where the animal lay. The animals were picked up within fifteen seconds of exposure so that if there was a dangerous amount of carbon monoxide in the region, they breathed at most only a small volume of the gas. It therefore seems highly improbable that this factor contributed in the least to the condition of the animals established by the exposure.

Preliminary experiments. Under this heading will be presented the results obtained with unanesthetized animals. The details of these experiments are presented in the protocols.

In the unanesthetized cats and dogs the most striking effect of exposure to the gun blast was the sudden onset of extreme fatigue. An animal previously alert, active and normal in every way was promptly changed into an exhausted, lethargic animal which roused when spoken to or when

touched; it would move about heavily for a few moments at a time and then settle down again in stupefied quiescence. Such animals neither ate nor drank; they could see and hear, exhibited no signs of external or internal injury other than the exhaustion just described; there was no bleeding at the nose or ears; stools were passed but there is no memorandum of voided urine.

In one animal (dog D) the respiration was disturbed early. The breathing was slow but deep and forced and appeared as if the respiratory muscles were imperfectly coördinated. At first this state of affairs was not noticeable and from a relatively inconspicuous onset it increased in prominence until the labored character was conspicuous. In the course of an hour this type of breathing had become extreme and air hunger was obvious; with each inspiration the snout was raised and the mouth opened. There was a definite grunt with each respiratory act. This condition prevailed presumably until death. The animal was found dead in the morning, about forty hours after exposure. Blood-stained froth was found in the mouth and nose. Cursory post-mortem examination revealed normal viscera except the lungs. These organs showed areas of red hepatization especially in the lower lobes, which were practically solidified. It was not believed at the time, however, that the pulmonary involvement was adequate to account for the extreme air hunger which had been observed.

A second animal (dog C) exhibited essentially the same picture throughout except that the respiratory disturbance was much later in onset, no abnormality being noted until twenty hours after exposure. This animal was comatose at the time it was sacrificed, three days after exposure. A third dog (dog A) exhibited the same extreme exhaustion and in addition was paralyzed in both posterior extremities. All the cats and dogs in the first series were exposed in heavy wooden crates which were frequently demolished by the gun blast. It was not thought that this dog was injured by a blow from the broken crate since there were no marks of external injury but it is quite possible that the paralysis had such an origin. In time the paralysis showed some improvement so that an effort was made to bring the hind legs under the trunk. Lethargy was extreme but up to the time of sacrifice there was no marked respiratory disturbance.

The last dog of the first series (dog B) returned from exposure exhausted, as were all the others, but on the second day was quite bright and drank some water. There was no evidence of respiratory disturbance and a note was made that recovery would probably occur.

The hemorrhagic areas of hepatization referred to above were noted in the cats and rabbits of this series as well. In fact, it was the single gross lesion found post mortem after exposure to air concussion due to gun blast or high explosive. The significance of this lesion will be discussed again. At this point it is proper to state that the injury to the lungs doubtless

contributed as a cause of death in these animals, if it was not the primary cause. The results indicate, however, that it was not the cause of the shock-like condition. The animals exhibited the marked lethargy and fatigue at once while respiratory distress and air hunger were relatively late in onset. Reference to the later experiments with anesthetized animals will show that the condition of shock in successful experiments as judged from the depression of the arterial blood pressure was present immediately (within five minutes) after exposure and while it was not easy to compare the degree of pulmonary injury in different animals, the conviction was established that the pulmonary involvement bore no direct relationship to the degree of shock. Furthermore in the high explosive experiments in which shock was never produced the lung injury was of extremest grade extending, in some cases, to occupy the greater part of the pulmonary tissues. Finally if the tissue destruction released a toxic substance it must have done so with remarkable rapidity to depress the circulatory function so promptly.

The results given here emphasize the greater resistance shown by dogs. Thus while dog B was not killed by exposure 14 feet in front of the ten-inch rifle, a cat (cat 4) and a rabbit (rabbit 4) exposed at the same time and place were instantly killed. It is possible that individual differences in resistance in the same species may likewise be found. If so, it would account for some variable results to be considered later. Whether such resistance is due to a greater vitality such as we might expect in the young or to a firmer bony structure protecting the tissues, the evidence at hand gives no clue.

Protocols of preliminary experiments on rabbits, cats and dogs

Cat 1. January 14, 1918.

2:40 p.m. Exposed 22 feet in front of 12 inch mortar. No evident effect.
2:50 Exposed 15 feet in front of 12 inch mortar.
3:05 Out of crate. Badly shaken but walks naturally; seems stupid. Hair slightly singed on back. Sight and hearing good.
3:45 Acts ill. Climbed out of box and settled near warm pipe.
5:45 Condition much the same. Comes when called. Hair ruffled and looks sick. Phlegmatic. Does not move when lightly pushed in head with foot.
8:30 a.m. (January 15.) Has cough. Now bright and active, now stupid and lethargic.
4:30 p.m. Quite normal although not lively.
7:00 a.m. (January 16.) Found dead. No autopsy.

Cat 2. January 19, 1918.

2:30 p.m. Exposed 15 feet in front of 10 inch rifle. Death occurred immediately.
Post mortem. Abdomen clear. Some fluid in pleural cavity. Lungs full of dark red areas. Cat has had cough and areas do not look fresh.

Cat 3. January 19, 1918.

10:30 a.m. Exposed 35 feet in front of 14 inch rifle firing reduced charge.
1:15 p.m. Exposed 35 feet in front of 14 inch rifle (uniformity charge). No apparent injury.
5:00 Eats, drinks and behaves normally. Purrs.

Cat 4. January 20, 1918.

9:55 a.m. Exposed 14 feet in front of 10 inch rifle. Instantly killed.

Rabbit 1. January 14, 1918.

1:30 p.m. Exposed 35 feet in front of 12 inch mortar. No appreciable injury. Nose and mouth slightly bloody.
1:45 Exposed 20 feet in front of 12 inch mortar. Animal jerking about when picked up. Two minutes to house by ears. Dead on arrival.

Post mortem. Blood-stained fluid in peritoneum. Lungs full of large hemorrhagic areas.

Rabbit 3. January 14, 1918.

2:08 p.m. Exposed 30 feet in front of 12 inch mortar. No evident injury.
3:30 Entirely normal.

January 19, 1918.

1:15 p.m. Exposed 35 feet in front of 14 inch rifle.
3:30 Eats freely and appears wholly undisturbed.

Rabbit 4. January 20, 1918.

9:55 a.m. Exposed 14 feet in front of 10 inch rifle. Instantly killed.

Dog A. January 19, 1918.

2:00 p.m. Exposed 15 feet in front of 10 inch rifle. Hind quarters paralyzed; cannot stand. Condition possibly due to injury from crate although no abrasions found on skin of back. Pupils dilated. Sees clearly.
3:30 Lying quietly. Looks about but will not move. Tired. Slow, exaggerated breathing.
4:00 Respiration 14, pulse 140.
5:00 Will not eat.
6:00 Respiration 16, pulse 140. Paralysis marked.
9:00 a.m. (January 20.) Respiration 12. Stupid.
12:15 p.m. Respiration 12.
4:00 Very quiet. Raises head weakly.
5:00 Respiration 12. Had moved length of room.
7:30 a.m. (January 21.) Respiration 12. Looks brighter.
4:30 p.m. Respiration 10. Lethargic.
7:30 a.m. (January 22.) No change. Sacrificed. Would probably have died. Had taken no water or food.

Dog B. January 20, 1918.

9:55 a.m. Exposed 14 feet in front of 10 inch rifle.
10:30 Respiration 42, pulse 96. Runs about but obviously affected.

12:15 p.m. Respiration 24, pulse 92. Quiet.
4:00 Respiration 30, pulse 160.
7:30 a.m. (January 21.) Respiration 20. Quiet. Looks brighter but has sniffles.
4:30 p.m. Respiration 26. Quiet.
7:30 a.m. (January 22.) No change. Sacrificed. Might have lived.

Dog C. January 20, 1918.

9:55 a.m. Exposed 14 feet in front of 10 inch rifle.
10:30 Appears very tired.
2:30 p.m. Respiration 36.
4:00 Respiration 38.
5:00 Respiration 22. Very quiet.
7:30 a.m. (January 21.) Has cough and expectoration. Occasional gasping respiration.
10:00 Gasping for breath and coughing.
11:45 Respiration 10. Condition worse.
1:00 p.m. Looks as if dying.
4:30 Respiration 16. Sitting up, gasping for breath.
7:30 a.m. (January 22.) Lying quiet. Noisy respiration. Sacrificed. Very sick. Would undoubtedly have died.

Dog D. January 19, 1919.

2:30 p.m. Exposed 15 feet in front of 10 inch rifle. Stunned. Breathes with jerk. Pupils dilated.
3:30 Walks about. Seems tired. Can see. Striking thing is slow labored breathing. Whole body moves and animal gasps for breath with open mouth although room is cold and animal is quiet. Respiratory muscles seem to lack coördination.
4:00 Respiration 20, pulse 104. Nausea and attempted vomiting.
5:00 Will not eat. Frothy clear saliva hanging from mouth.
6:00 Respiration 26, pulse 140. Appears slightly better.
9:00 a.m. (January 20.) Respiration 50, pulse 134. Walks easily. Occasional sighing grunt. Respiration jerky. Nose lifted and mouth opened with each respiration.
4:00 p.m. Respiration 38, pulse 72. Noisy, grunting respiration.
7:30 a.m. (January 21.) Found dead.

Post mortem. Abdomen clear. Lower lobes of lungs hepatized. Upper lobes slightly affected. No fluid in pleural cavity. Lung involvement not extensive enough to explain respiratory distress.

Animal was perfectly well and lively before exposure.

Experiments with frogs. Sixteen frogs were used at various times in these experiments. Although unsatisfactory for certain aspects of the subject, they contributed some valuable information. The protocols show that the lungs were easily ruptured, air bubbles being repeatedly found in the body cavity. This observation indicates that it is the positive wave of pressure and not the negative wave following, as has been suggested, which is the causative factor in the injury for it is wholly improbable that lowered pressure could distend these organs sufficiently

to cause their rupture. On the other hand, a sudden elevation of pressure retarding the expulsion of air through the trachea and forcibly compressing the body walls could readily rupture these delicate organs.

The hypothesis has been advanced (8) that concussion shock is due to air emboli liberated in the blood stream, as nitrogen gas forms emboli in caisson disease. It is improbable that the extremely sudden changes in pressure associated with air concussion could establish such a condition and careful search for air emboli in the heart, large veins and mesenteric vessels in frogs failed wholly to support the conception. The same negative results were obtained in dogs.

The pressure adequate to kill a frog is apparently in the neighborhood of fifteen atmospheres. This does not differ much from that necessary to cause death in dogs, although, as was stated earlier, the duration of the pressure is apparently a conflicting and unknown factor in these results.

No evidence could be obtained that the peripheral neuro-muscular mechanism was in any way injured. Stimulation of the sciatic nerve in moribund frogs gave a ready muscle response at various times after exposure. Likewise, except in the period immediately after exposure, coördinated reflex movements could be obtained as in a reflex preparation. This result points not only to the absence of injury in the peripheral neuromuscular and receptive mechanisms but also to the absence of injury to the centers in the cord. The reflex time was essentially unaltered although movements when elicited were sometimes decidedly incoördinated and spastic. An examination of the central nervous system in one of these frogs by Major C. J. Herrick revealed no obvious lesion; there were indications of venous congestion but no hemorrhages or degenerative processes were found although the animal was not sacrificed for examination until some days after exposure.

Attention should be called especially to the protocol of the frog exposed January 14, 1918, because of the symptoms exhibited and the time it lived after exposure. This frog apparently suffered no pulmonary injury adequate to cause death. It was sacrificed nine days after exposure for histological study. The sluggishness of this frog suggests the languor seen in the unanesthetized dogs. Its inability to hop and to make nice movements; its quick fatigue and failure to respond to repeated insulting stimulation; its deviation to one side in crawling all suggest a central nervous system injury and yet Major C. J. Herrick, who made a somewhat hurried histological study of the brain and cord noted no obvious abnormalities. It is certain, in any case, that there were no well-defined hemorrhagic areas or areas of degeneration visible in the nervous system. It is only fair to Major Herrick to state that he was not in a position to make a thorough examination of these tissues and while the experimental observations appear to conflict with his results, it does not follow that he would

not have found cytological abnormalities had he been able to study the tissues carefully. The fact, however, that no outstanding microscopic lesions were apparent in connection with the symptoms of disordered locomotion and sensory response is a point of very great interest. It is improbable that this frog presented a psychosis akin to "shell shock," yet it exhibited a clinical picture similar in many respects to that described for human beings classified and treated as "shell shock" cases. It is thus open to question if the war psychoses which developed after exposure of individuals to the detonation of high-explosive shells were *all* purely psychic in character in that no material injury was done the central nervous system.

In this connection reference may be made to the experience with two other frogs. These animals were unintentionally exposed in a pasteboard box on a neighboring gun-carriage some thirty feet to the side and ten feet behind the muzzle of the gun at the time the frog just discussed was exposed. When these frogs were examined shortly after exposure, a note was made that they were "obviously injured." They showed spastic, slow and useless movements of the limbs. They could not turn over if placed on their backs. The legs were held flexed with the back arched and the head resting on the ground. If suspended by a fore leg the trunk and hind legs slowly relaxed and hung supine, the animal making no effort to escape. An hour and a half later they were observed to sit and to move naturally but still righted themselves with difficulty if placed on their backs. In four hours, however, they were entirely normal and showed no subsequent ill effects of the exposure.

Again it may be assumed that we are not dealing with a psychosis. Rather it would seem that the disturbance set up in the body cells and particularly in the cells of the brain and cord upset the normal function. As distinguished from the first frog, the injury done these two was transient in character and passed off in a few hours, while the injury done the first frog was severe enough to leave a lasting effect, an effect, however, not explained by the post-mortem anatomical changes.

In one experiment a series of seven frogs was exposed at twenty-two inch intervals to the detonation of five pounds of trinitrotoluene (high explosive). Those separated from the charge by a distance of eighty-eight inches or more were practically unaffected. Twenty minutes after exposure they were active and apparently well. The respirations were fast and deep, otherwise there was nothing to distinguish them from unexposed frogs. All of them lived and showed no subsequent ill effects. Those which were exposed within a radius of sixty-six inches were obviously injured since none of them exhibited reflex movements up to an hour after exposure.

These frogs were opened to study the condition of the abdominal veins. Comparison was made with a normal frog killed by pithing and it was found that all of them showed an obvious distention of the large veins. The hearts were beating; the ventricles were shrunken and pale but the auricles were so markedly distended that at first the feeble contractions could not be recognized. Clearly in these frogs the blood was pooled in the great veins. The "Klopf-versuch" of Goltz was immediately brought to mind by this picture.

Another frog was similarly examined alongside of a normal control, six hours after exposure to the twelve-inch mortar. In this animal the auricles were not distended and the cardiac function appeared normal. The veins were somewhat distended although the picture was not striking.

The significance of the engorgement of the veins in frogs shortly after exposure will be emphasized in connection with the dog experiments, in which a similar engorgement was noted immediately after exposure but which apparently subsided in the course of some hours. In the dogs this engorgement was accompanied by a low venous blood pressure indicating a loss of venous tone. The fact that the engorgement subsided in a few hours is of interest in connection with statements made by surgeons in the base hospitals that shocked cases exhibited no engorgement of the abdominal veins. It is possible that in experimental animals as in the clinical cases a transudation of blood plasma, associated with the depression of all the body functions incident to the low blood pressure, may account for the absence of venous engorgement some hours after shock has been established.

Protocols of experiments on frogs

Frog 1. January 14, 1918.

11:25 a.m. Exposed 15 feet in front of 12-inch mortar.

1:00 p.m. No spontaneous movements. Lies prone with head resting on ground and limbs extended. Feeble, incomplete hind-leg reflexes.

3:30 Reflex responses more active. Still no spontaneous movements. Head rests on ground but limbs are now drawn up to body.

5:45 Strong stimulation causes violent intentional movements which are poorly coördinated; both eyes open wide. No eye-lid reflex. Does not assume normal position.

8:30 a.m. (January 15.) Position somewhat more normal but does not hold up head. No respiratory movements visible. Reflex time of withdrawal of toe from dilute sulphuric acid roughly same as control frog (5 seconds) but more labored as if legs were heavy; movement of withdrawal wide and ataxic. Response to mechanical stimulation (pinching toe) much slower than normal frog. Strong pinching for some time produced wide, disordered kicks very different from the quick, orderly and purposeful lifting of the foot to the flank by the normal control frog. Slight edema in left knee.

11:15 a.m. Improvement apparent. Position better. For first time is able to turn over if placed on back. In doing so movement is always to same side. Holds head up after period of excitement and respiratory (throat) movements can be seen for brief time.

4:30 p.m. Will turn over sometimes, but not regularly, if placed on back. No respiratory movements. Heart beat visible. If stimulated when lying on ventral surface makes attempt to progress; movements then awkward and circles to right. Will not move far and further stimulation is without effect.

8:00 a.m. (January 16.) Distinctly less well. Blood-stained urine. Turned over once in six separate tests. Holds head up for brief periods. Strong stimulation does not elicit attempts to move. Reflex lifting of feet on mechanical stimulation very feeble. Heart beats visible.

2:00 p.m. Able to turn over once.

4:30 Not so well. Could not run over and would make but one attempt. Quick but feeble reflex movement of foot. Holds head up only momentarily. No progression. Heart beats visible.

7:45 a.m. (January 17.) Bloody urine. More phlegmatic.

4:00 p.m. Apparently better. Water-clear urine. Turned over three times. Thereafter would make no effort in response to strongest stimulus.

7:45 a.m. (January 18.) Blood-stained urine. Otherwise no change.

7:30 (January 22.) No change.

1:00 p.m. (January 23.) Slightly more active. No respiratory movements. Can turn over but is phlegmatic. Cannot hop but crawls awkwardly and always circles to right. Does not hold up head except after irritation. Appears sleepy.

Turned over to Major Herrick. Major Herrick subsequently reported that no gross lesions or areas of hemorrhage were found in the brain or cord. Ordinary histological examination of these tissues likewise failed to reveal any evidences of injury

Frogs 2 and 3. January 14, 1918.

11:25 a.m. Unintentionally exposed in pasteboard box on gun-carriage 10 feet behind and 30 feet to side of muzzle of 12-inch mortar at time frog 1 was exposed. Immediate inspection showed them obviously injured. Continuous spastic, helpless and slow movements of limbs. Cannot turn over if placed on back. Hold legs flexed with back arched and head resting on ground. Suspended by fore leg, trunk and hind legs slowly relax and hang supine.

1:00 p.m. Both frogs move and sit naturally. Turn over with difficulty but are clearly improved.

3:30 Entirely normal in appearance and behavior.

7:30 a.m. (January 15.) Condition normal. Observations discontinued.

Frogs 4 and 5. February 13, 1918.

4:20 p.m. Exposed 25 feet in front of 10-inch rifle. One was opened at once. No air emboli found in heart or large vessels. Other was returned to laboratory. Sluggish. Breathes. Heart beats visible. Struggles in hand. Turns over if placed on back. Not roused by mechanical but electrical stimulation causes frog to hop.

4:55 Hops about without urging.

7:00 a.m. (February 14.) Perfectly well.

Frog 6. February 13, 1918.

4:20 p.m. Exposed 35 feet in front of 10-inch rifle. Was blown about 100 feet.
4:30 No superficial injury. No respiration. Heart beats not seen. Twitching of toes noted.
4:45 Both hind legs twitch. Pinching toes causes feet to be lifted. Slight effort to turn over when placed on back.
7:00 a.m. (February 14.) Found dead.

Frog 7. February 14, 1918.

10:25 a.m. Exposed directly beneath and 2 feet behind muzzle of 12-inch mortar.
10:35 Breathes. Heart beats visible. Sluggish but turns over if placed on back.
11:30 Hops weakly.
3:30 p.m. Almost dead. Opened. Lungs and stomach filled with blood. Heart beating. No air emboli found in heart or blood vessels. Compared with normal frog pithed and opened at this time, the veins are somewhat distended.

Frog 8. February 14, 1918.

10:25 a.m. Exposed 2 feet in front and 2 feet to side of 12-inch mortar.
10:35 Not breathing. Heart beats visible. Opened. Ventricle pale and empty. Auricles distended and full of blood. Auricles contract but blood is not moved. Veins distended. Sciatic nerve exposed and stimulated (coil 10); muscles respond to single make and break and tetanic stimuli. No air emboli in blood vessels. Preserved in formalin.

Frog 9. February 14, 1918.

10:25 a.m. Exposed 5 feet in front of 10-inch mortar.
10:35 Not breathing. Heart beats not visible. No muscular twitching. Flaccid. Appears dead.
7:00 (February 15.) Dead and stiff.

Frogs 10, 11, 12, 13, 14, 15 and 16. March 6, 1918.

1:48 p.m. Exposed to detonation of 5 pounds trinitrotoluene;—22, 44, 66, 88, 110 and 132 inches respectively from charge.

Frog 10 opened at 2:45 p.m. Body cavity filled with bloody froth. Lungs hemorrhagic and ruptured. Heart beating; dilated at first but shortly assumed normal appearance. No reflex movement elicited. Veins distended. No air emboli.

Frog 11 opened at 2:45 p.m. A little bloody froth in body cavity. Lungs hemorrhagic. Heart beating. No reflex movements elicited. Veins distended. No air emboli.

Frog 12 opened at 2:45 p.m. Bloody froth in body cavity. Veins distended. No air emboli.

Frog 13 opened at 2:23 p.m. Lungs hemorrhagic. Small amount of coagulated blood in body cavity. Heart beating. Ventricle pale and contracted. Auricles and veins distended. No air emboli.

Frogs 14, 15 and 16 observed at 2:10 p.m. Alive and active. Breathing fast and deep. Observed at 4:30 p.m. next day. Appear perfectly well.

Frogs 17, 18, 19, 20 and 21. March 18, 1919.

4:30 p.m.	Exposed at points in front of 8-inch rifle such that the concussion pressures as measured with the Baroscope were:
	Frog 17. 142 pounds per square inch.
	Frog 18. 152 pounds per square inch.
	Frog 19. 237 pounds per square inch.
	Frog 20. 129 pounds per square inch.
	Frog 21. 180 pounds per square inch.
4:50	Frog 17. Hops well. Turns over.
	Frog 18. Turns over. Tangles legs. Will not move when stimulated.
	Frog 19. Turns over. Tangles legs. Hops feebly.
	Frog 20. Turns over. Does not tangle legs. Hops feebly.
	Frog 21. Turns over. Hops well.
7:00	All appear well except frog 19, which is dead. Preserved in formalin.
7:30 a.m.	(March 19.) All appear well.

Experiments with high explosive. Dogs 12, 13, 17, 18 and 19 were exposed to the detonation of high explosive (trinitrotoluene). The protocols show that the method was inadequate for the production of shock. One animal (dog 18) showed a fall in arterial blood pressure from 114 mm. to 74 mm. Hg and a fall in venous blood pressure (femoral) from 8.75 cm. to 6.25 cm. H_2O, a half-hour after exposure. An hour later, however, the arterial pressure had risen to 100 mm. Hg and the venous pressure to 8.0 cm. H_2O. This was the nearest approach to shock obtained in the series. The animal was sacrificed an hour and a half after exposure. The abdomen was entirely normal. Blood was present in the left pleural cavity and the lungs were extensively hemorrhagic.

The notes on these experiments agree in emphasizing the injury to the lungs. The laceration of the tissue was extensive and the hemorrhagic condition widespread throughout the organs. It was very evident that the destruction of lung tissue was much greater than in animals exposed to gun blast. An explanation of this phenomenon is not at hand unless it be the suddenness with which the rise of pressure occurs.

The very considerable involvement of lung tissue in these animals, none of which exhibited the characteristics of shock, is significant as indicating that the pulmonary lesion produced by air concussion is not the primary cause of shock. If this were the case these animals with profound lung injuries should have exhibited marked symptoms of low blood pressure. As it was, none of them showed typical shock. The observation indicates further the probability that tissue injury, in the sense of releasing toxic substances like histamine into the circulation, is not of primary importance in the production of concussion shock (9).

It is not to be concluded from the foregoing discussion that there is anything peculiar about the air concussion administered in these experiments with high explosive. It may be that the duration of the phase of

high pressure is brief as suggested by the sound produced, but the most probable reason for the failure of these experiments lies in the fact that the pressure is dissipated with great rapidity. Thus a charge of four pounds of trinitrotoluene detonated on the soft sand forms a crater about one foot deep and three feet in diameter. Beyond the crater's edge the area of pressure adequate for the purposes in hand must be very narrow. Within the crater the pressure is excessive. To place a large animal in the area of adequate pressure is almost impossible. Doubtless had it been justifiable to use charges in excess of four pounds the results would have been more satisfactory in that the area of adequate pressure would have broadened. All the circumstances surrounding the work, however, made it advisable to discontinue this line of attack.

Protocols of experiments on dogs exposed to high explosive

Dog 12. *March 7, 1918.* *Male, large.* One grain morphia hypodermically.

Dog exposed 2 feet from 2 pounds T.N.T.
Instant death. Thrown 20 feet. Hind limbs shattered and abdominal wall ruptured. Skin burned. Lungs brightly hemorrhagic. Huge blood-clot in thoracic cavity. No specimens preserved.

Dog 13. *March 9, 1918.* *Male, large.* One grain morphia hypodermically.

8:15 a.m. Dog exposed 4½ feet from 2 pounds T.N.T.—one foot outside of crater.
No effect upon arterial pressure or heart-rate.
Respirations slower and deeper.
10:25 Sacrificed.
Post mortem. Lungs diffusely hemorrhagic. No specimen preserved.

Dog 17. *March 13, 1918.* Morphia-ether anesthesia.

9:15 a.m. Exposed 4 feet from 4 pounds T.N.T.
Bloody froth from trachea on return to laboratory.
9:43 Dead.
Post mortem. Copious hemorrhage from nose 9 minutes after death. Lungs practically all hepatized. About 25 cc. bloody fluid in pleural cavity. No specimens preserved.

Dog 18. *March 13, 1918.* Morphia-ether anesthesia.

1:10 p.m. Arterial pressure 114 mm. Hg.
Femoral venous pressure 8.75 cm. H_2O.
1:57 Exposed 4 feet from 4 pounds T.N.T.
2:30 Arterial pressure 74 mm. Hg.
Femoral venous pressure 6.25 cm. H_2O.
3:20 Arterial pressure 100 mm. Hg.
Femoral venous pressure 8.0 cm. H_2O.
3:30 Sacrificed.
Post mortem. Lungs extensively hemorrhagic. Blood in left pleural cavity. No specimens preserved.

Dog 19. March 14, 1918. Chloretone anesthesia.

2:03 p.m. Arterial pressure 90 mm. Hg.
Femoral venous pressure 8.5 cm. H_2O.

2:27 Exposed 41 inches from 4 pounds T.N.T. Animal placed back to charge. Snout wrapped in towel and pushed into 3 inch shell.

2:55 Arterial pressure 90 mm. Hg.
Femoral venous pressure 7.5 cm. H_2O.

3:00 Sacrificed.
Post mortem. Posterior lower lobes of lungs hepatized. No specimens preserved.

TABLE 1

	DOG NUMBER	GUN	DISTANCE FROM MUZZLE	AIR PRESSURE IN POUNDS PER SQUARE INCH
			feet	
Shock	1	10″ rifle	18	
	2	10″ rifle	18	
	5	10″ rifle	18	
	7	12″ rifle	10	
	14	12″ rifle	20	
	28	12″ rifle	20	267
Killed instantly	15	12″ rifle	10	
Incomplete shock	10	12″ rifle	10	
	11	12″ rifle	50	
	20	10″ rifle	13	
	21	10″ rifle	15	
	26	10″ rifle	15	67.6, 146, 319.5
	27	10″ rifle	15	281
No shock	6	10″ rifle	15	
	8	12″ rifle	5	
	16	12″ mortar	17	
	22	10″ rifle	12	135
	24	12″ mortar	11	388
	29	8″ rifle	18	

Experiments with the gun blast. A series of nineteen dogs was used in these experiments. Six of them were not regarded as showing shock, six showed incomplete shock, one died before observations could be made and six exhibited primary shock. These numbers have no comparative value since the animals were exposed under different circumstances, as shown in the accompanying table (table 1). The variations in the results, under conditions of pressure which were assumed to be constant, discussed earlier in this paper, are brought out in this table. The pressure adequate to produce shock was found from ten to twenty feet in front of the ten and

twelve-inch rifles. But the results are not consistent. Thus, one animal suffered characteristic shock ten feet in front of the twelve-inch rifle, another animal was killed instantly and a third was incompletely shocked in the same position. The protocols do not indicate whether or not the powder charge varies but the drift in the point of maximum pressure so clearly brought out in Miller's results is probably the chief factor responsible for these variations. If the point of maximum pressure lies at a given point at one time, there is no assurance that it will be found there at another time and although the average of a number of pressure observations would give the probable point of maximum pressure, successful experiments could be consistently had only with the exposure of several animals at a time. Such a procedure as this was, however, not feasible.

The table also gives the pressures to which some of the dogs were exposed. The data are insufficient to warrant a positive statement as to the concussion pressure requisite to produce shock. From the figures one may judge, however, that approximately two hundred and seventy-five pounds per square inch (18 to 19 atmospheres) are necessary to establish primary shock in the case of the ten- and twelve-inch rifles. It is interesting to note that the twelve-inch mortar, even when a pressure of three hundred and eighty-eight pounds per square inch was delivered, did not produce shock. Miller, in repeated observations, found that this gun, which is mounted somewhat nearer the ground than the rifles, gave uniformly high pressures. The only explanation which seems to accord with Miller's observations and my own results is, as has been stated, that the duration of the high pressure phase, in the case of the mortar, is insufficient to overcome the physical resistance of the tissues. This hypothesis is supported, so far as the figures go, by the fact that two hundred and eighty-one pounds before the ten-inch rifle was less effective than two hundred and sixty-seven pounds before the twelve-inch rifle, the latter presumably having the longer phase of high pressure.

It is scarcely probable that the physical resistance of the tissues, if this be a factor, is associated with the soft abdominal and lower thoracic regions of the body and it is therefore open to question if the condition of shock in these experiments is not primarily associated with brain concussion. The vacuolarization of the brain cells and the disintegration of the Nissl substance seen in stained sections of the nervous tissue accords with this concept although this view must be regarded at the present time as pure hypothesis.

Arterial blood pressure. An arterial blood pressure of half the normal or less was taken as signifying a condition of shock. That shock may be present without a persistent low arterial pressure is possible but that an abnormally low pressure can persist without shock is wholly improbable. Hence the experiments were primarily classified according to the effect

produced upon the arterial blood pressure, as shown in table 2. In accordance with this criterion, six animals exhibited shock, seven exhibited incomplete shock and five were unaffected.

Our interest is centered primarily in the six cases of complete shock. The suddenness with which the fall in arterial pressure occurs is most striking. In dog 2, for example, the control observation gave an arterial pressure of 150 mm. Hg. Seven minutes after exposure the pressure was 50 mm. Hg. The pressure remained unchanged at this level until just before death, four hours after exposure. In dog 1 the control reading was 84 mm. Hg. This is a low value due to the use of chloretone anesthesia. Ten minutes after exposure the pressure was 36 mm. Hg. In the course of

TABLE 2

Arterial blood pressure before and after exposure

SHOCK					INCOMPLETE SHOCK					NO SHOCK				
Dog number	Gun	Distance	Before	After	Dog number	Gun	Distance	Before	After	Dog number	Gun	Distance	Before	After
		feet					*feet*					*feet*		
1	10″ rifle	18	84	36	8	12″ rifle	5	138	126	6	10″ rifle	15	110	112
2	10″ rifle	18	150	50	10	12″ rifle	10	100	70	16	12″ mortar	17	130	130*
5	10″ rifle	18	70	46	11	12″ rifle	50	130	90	22	10″ rifle	12	90	90
7	12″ rifle	10	136	66	20	10″ rifle	13	142	134	24	12″ mortar	11	90	105
14	12″ rifle	20	130	15	21	10″ rifle	15	145	90	29	8″ rifle	18	110	105
28	12″ rifle	20	105	40	26	10″ rifle	18	85	90					
					27	10″ rifle	15	120	98					

* Two exposures.

three hours it rose to 50 mm. Hg and held for an hour. Thereafter it fell slowly until the animal died ten and a half hours after exposure.

This sudden drop in arterial pressure to shock level is in remarkable contrast to the slow fall developed by most laboratory methods of producing shock, i.e., visceral trauma, injection of epinephrin, partial occlusion of aorta or inferior vena cava, etc. On the other hand hemorrhage, injection of peptone, histamine and other toxic substances and the anaphylactic reaction give an equally sudden drop in arterial pressure. The usual outcome of the latter procedures is, however, death or restoration of blood pressure in a compartively short time. Hence the shock condition produced by air concussion differs strikingly from shock produced by any other means, and it is probable that it has an independent etiology.

Another striking point in connection with the arterial blood pressure was brought out in this series of experiments. The animals which were shocked

showed the lowered blood pressure at the first observation within ten minutes of exposure. Animals which were not thus immediately thrown into shock but which were returned to the laboratory with a good arterial pressure did not develop shock in a period of two hours or more. This statement is true likewise of the animals exposed to high explosive. In other words, concussion shock is of instantaneous onset and is quite unrelated to progressive tissue changes. This points again to a purely nervous etiology and may be taken to indicate the improbability of successful treatment.

The sole gross lesion in concussion shock is the pulmonary injury. This might cause internal hemorrhage or, as the result of tissue destruction, release into the blood stream a toxic substance. That the explanation of concussion shock is not related to either of these factors is clearly indicated by the failure to produce shock by the detonation of high explosive which developed the most extreme pulmonary injury. If the hemorrhage or tissue destruction were the primary causative factor, the experiments with high explosive should have produced shock. This was not the case and furthermore, in the animals exposed to gun blast, there was no apparent parallelism between the degree of pulmonary injury and the depression of the arterial blood pressure.

Venous blood pressure. The venous pressure determinations in centimeters of water are presented in table 3. The figures for the venous pressure as for the arterial pressure given in this table represent the readings made immediately on return to the laboratory. Briefly this table shows that in five animals which did not show shock the venous pressure fell in two, rose in two and remained unchanged in one; that in five animals in incomplete shock it fell in two and rose in three and that in six animals in complete shock it fell in four and rose in two. The results thus indicate that the venous pressure is especially depressed in the cases of shock. The two exceptions to this statement, furthermore, may be readily explained. One of them, dog 14, lived but a very short time and was in a dying condition when the venous pressure determination was made. The other, dog 7, was used in an effort to determine the "effective venous pressure" as suggested by Wiggers (10). The method consists in connecting a thoracic sound with the top of the venous pressure manometer in order to record the pressure effective in the venous filling of the heart. It was not suited to the present work, however, because the thoracic wound very probably admitted air when the animal was exposed and it is assumed that the rise in venous pressure noted in the animal under discussion was due to a disturbance of intrathoracic negative pressure. If these explanations are allowed, it follows that there were no exceptions to the statement that concussion shock is associated with a lowered venous pressure.

TABLE 3

Arterial and venous blood pressures before and after exposure

SHOCK							INCOMPLETE SHOCK							NO SHOCK						
Dog number	Arterial		Venous				Dog number	Arterial		Venous				Dog number	Arterial		Venous			
			Femoral		Jugular					Femoral		Jugular					Femoral		Jugular	
	Before	After	Before	After	Before	After		Before	After	Before	After	Before	After		Before	After	Before	After	Before	After
1	84	36	6.5	2.5	2.5	−6.0	8	138	126			4.5	8.5	6	110	112	4.5	4.0	0.5	−1.0
2	150	50	15.5	5.75	4.5	−3.0	10	100	70	6.25	15.0	3.25	10.0	16	130	130	5.25	5.25		
5	70	46	2.75	4.0	1.0	−0.5	11	130	90	8.25	5.0	0.0	−1.5	22	90	90	5.7	5.5		
7	136	66			3.5	5.75	20	142	134	2.5	5.0			24	90	105	9.3	10.5		
14	130	15	3.5	15.0			21	145	90					29	110	105	2.5	7.0		
28	105	40	10.5	9.8			26	85	90											
							27	120	98	10.0	8.8									

The femoral venous pressure was determined in five of the shocked animals. The pressure was recorded as lowered in three and raised in two of them. This rise has just been explained in one, dog 14, as being due to premortal circulatory failure. In the other, dog 5, the jugular venous pressure fell. This animal had been operated on for inspection of the abdominal viscera and it is not improbable that this procedure may have resulted in mechanical interferences with the organs such that the normal venous flow was thereby impeded, causing the femoral pressure to rise.

The *jugular venous pressure* was determined in four of the shocked animals. In all but one the pressure fell. This animal was submitted to Wiggers' procedure for determining the "effective venous pressure" and the rise in pressure observed is ascribed, as has just been stated, to a disturbance of the intrathoracic negative pressure.

It may be stated, therefore, with conviction, that air concussion causes a fall in venous pressure when the concussion is adequate to produce shock. This cannot be secondary to the fall in arterial pressure because arterial pressure falls acutely only as the result of an inadequate venous pressure, cardiac failure or vasomotor collapse. In vasomotor collapse the circulating blood floods into the veins and a rise of venous pressure is recorded. A similar rise of venous pressure results from cardiac failure. A venous pressure inadequate to fill the heart in diastole might follow hemorrhage or a loss of tone in the veins and capillaries. Post-mortem, these animals show clear evidence of ruptured pulmonary blood vessels. This lesion may, however, be present in an extreme grade without shock and without a low venous pressure, and in any case it is extremely improbable that the letting of an amount of blood equivalent to that segregated in the injured areas of the lungs would be sufficient to establish a condition of circulatory failure. Likewise the injury done the lungs could not produce the fall in arterial pressure by blocking the circulation without at the same time raising the venous pressure.

A decrease in blood volume due to transudation of plasma into the tissue would undoubtedly produce a circulatory condition such as that described, but it is scarcely conceivable that it could be developed within so short a time—seven minutes. That transudation of plasma occurs, but not as an acute process, is indicated by the appearance of the abdominal veins. These veins, as will be described later, appear engorged at first but subsequently shrink and become inconspicuous.

The independence of the venous pressure with respect to the arterial pressure is emphasized in the protocol of dog 1. The readings are given in table 4. It will be noted that the arterial pressure was low immediately after exposure, rose for a time, remained stationary and then fell. During this period, approximately seven hours, the venous presure tended to rise as the arterial pressure rose. The changes in arterial pressure preceded

changes in venous pressure but it is difficult to see a direct relationship between them. It is much more probable that the regulation of the venous pressure depends upon an independent mechanism.

The observations on the behavior of the venous pressure which have been discussed raise two points of interest. First, there is the fact that the venous pressure is independent, in part at least, of changes in arterial pressure. This lends support to the newer view that the venous circulatory bed functions apart from the arterial control and is subject to a special nervous and chemical regulation. Second, there is the fact that the venous pressure is lowered even in extreme collapse of the arterial pressure. Except by the loss of circulating plasma this condition could only be explained in our present state of knowledge by a loss of nervous tone resulting in a pooling of blood in the veins or capillaries. The evidence for such a loss of tone is found in the observations to be presented shortly that the

TABLE 4

Arterial and venous pressures in dog 1

		TIME						
		9:15	10:25	11:35	1:30	2:30	4:00	4:40
Arterial pressure in mm. Hg		84	36	46	50	50	44	42
Venous pressure in cm. H_2O	Jugular	2.5	−6.0	−6.5	−6.25	−5.75	−6.0	−1.5
	Femoral	6.5	2.5	−1.0	1.0	3.75	2.25	

Exposed at 10:12 a.m.
Death at 8:43 p.m.

abdominal veins even when registering a lowered pressure are distended and engorged. This condition of affairs supports the older conception that shock may be associated with a vascular collapse such that the circulating blood is pooled in the veins. Since the blood in the latter is under a lowered tension it follows that there must be concomitant loss of venous tone. The vascular collapse is thus not limited to the arterial bed in accordance with the older conceptions of shock, but involves an active loss of venous and perhaps of capillary tone.

Heart. No particular attention was directed to the cardiac function. The rate of the heart beat was increased in the majority of cases. This change probably represented the usual reaction to low blood pressure since the peripheral vagus mechanism was in function as shown by the fact that peripheral vagus stimulation readily caused inhibition. Post-mortem inspection showed the valves to be intact. The cardiac muscle, examined histologically, showed no definite pathological changes and the heart was not embarrassed by transitory elevations of the arterial pressure produced by stimulation of the vasomotor center.

Respiration. The respiration was usually fast and shallow. Whether or not the respiratory volume was affected could not be determined by the methods employed.

Alkaline reserve. The alkaline reserve was determined in five animals. Specimens of arterial blood were drawn and, after agitation with potassium oxalate crystals, centrifuged. The plasma thus collected was kept on ice until delivered to Doctor Van Slyke at the Rockefeller Hospital for analysis. The plasma thus obtained after exposure of the animals was perfectly clear, indicating that no destruction of the red blood cells occurred as a result of the air concussion.

Table 5 shows the results obtained together with the arterial pressures at the time the blood samples were drawn. Dog 7, which exhibited the typical picture of primary shock, gave a normal value of five minutes after exposure. An hour and three quarters after exposure the alkaline reserve

TABLE 5

Arterial pressure and alkaline reserve

	DOG 7			DOG 8				DOG 10		DOG 11		DOG 28		
Time	1:30	2:35	4:15	3:35	4:54	5:45	6:45	9:25	10:13	8:20	10:20	8:40	10:05	10:58
Arterial pressure	136	66	85	138	126	110	126	100	70	130	90	100	40	45
Alkaline reserve	69.1	69.1	43.8	39.5	46.2	49.0	50.5	43.5	47.2	39.7	33.1	56.4	43.8	38.9

Dog 7 exposed at 2:30.
Dog 8 exposed at 4:46.
Dog 10 exposed at 10:09.
Dog 11 exposed at 10:11.
Dog 28 exposed at 9:53.

had fallen from 69.1 per cent to 43.8 per cent. Dog 8, which was not in shock, showed an increase in alkaline reserve. Dog 11, likewise not in shock, showed a definite decrease nine minutes after exposure. Dog 28, severely shocked, showed a decrease twelve minutes after exposure. Dog 10 was so severely injured by the exposure that it died in a half-hour. The rate of respiration was reduced from 24 to 9 per minute and the venous pressure was raised. The animal was moribund at the time the blood sample was drawn and therefore gives no data of value in the present connection. The lungs were found extensively hemorrhagic and it is assumed that the arterial pressure remained elevated because of asphyxiation.

These results do not permit of unqualified deductions. It is clear, however, that a depletion of the alkaline reserve is not an essential feature of concussion shock since dog 7 gave a normal value five minutes after an exposure which caused a drop in arterial pressure from 136 to 66 mm. Hg. Penfield (11) found that maintenance of a low arterial pressure by hemor-

rhage promptly reduces the alkaline reserve and we may believe that in the present series of animals the low arterial pressure quickly disturbs the tissue oxidations and therewith the ratio $\frac{H_2CO_3}{NaHCO_3}$. Unless the blood specimen is obtained immediately after exposure, these changes will develop and the alkaline reserve will show a reduction. The fact, however, that when the blood is drawn soon enough after shock is established this sign of acidosis is not found indicates that acidosis is not a primary factor in concussion shock.

Catalase. Burge and others have called attention to the intimate relation of catalase, an enzyme present in the blood and tissues capable of

TABLE 6

Arterial pressure and blood catalase

	DOG 2			DOG 5			DOG 6		DOG 7			DOG 8			DOG 10	
Time	10:00	11:35	1:00	2:40	3:28	4:30	12:55	2:00	1:30	2:35	3:15	3:35	4:54	6:45	9:25	10:13
Arterial pressure	150	50	54	70	46	40	106	112	136	66	90	138	126	126	100	70
Catalase	165	128	95	16.7	9.8	16	161	165	24	13	19	9	9.2	7.5	12	11

	DOG 11		DOG 14		DOG 21		DOG 22			DOG 26		DOG 28		
Time	9:32	10:20	1:30	2:36	11:00	11:35	9:45	11:11	11:35	9:05	11:45	8:30	10:00	10:50
Arterial pressure	125	90	130	15	145	90	90	90		85	35	100	40	50
Catalase	14.5	14.5	14.5	15.4	57	58	60.8	102	91.2	45.5	24.9	47	45.5	48.9

Dog 2 exposed at 11:05.
Dog 5 exposed at 3:21.
Dog 6 exposed at 1:44.
Dog 7 exposed at 2:30.
Dog 8 exposed at 4:46.
Dog 10 exposed at 10:09.
Dog 11 exposed at 10:11.
Dog 14 exposed at 2:29.
Dog 21 exposed at 11:26.
Dog 22 exposed at 11:00.
Dog 26 exposed at 11:00.
Dog 28 exposed at 9:53.

liberating oxygen from hydrogen peroxide, to numerous functional processes. In the case of shock by hemorrhage or intestinal manipulation the catalase content of the blood is low (3). It was not expected that the catalase would show a reduction as a primary factor in concussion shock. It seemed worth while, however, to put the matter to experimental test.

The results obtained are brought together in table 6. The hydrogen peroxide used was not neutralized and different makes of the reagent were employed in different experiments which doubtless accounts for the wide variations in the results as shown. But in each experiment the reagent from one bottle only was used. The figures in the table have not been reduced to constant atmospheric conditions since relative values only

were required. It will be observed that the figures are quite inconsistent. Dogs 2, 5, 7 and 26, all shocked animals, show a drop in catalase paralleling the arterial pressure. Dog 14, also a shocked animal, shows a rise and dog 28, in extreme shock, shows no change. The inconsistencies may be explained in part by the very great difficulty in obtaining accurately measured blood samples by the use of an hypodermic syringe. The single experiment in which confidence was felt in the accuracy of these measurements was that with dog 28, in which the blood was measured in a pipette, and in this case there was no change in the catalase immediately and an hour after exposure. It may be concluded, therefore, that a reduction of the blood catalase is not a primary factor in concussion shock. The majority of the experiments seem to indicate, however, in accordance with Burge's work, that the catalase is reduced in conditions of low blood pressure.

Condition of medullary centers. Air concussion, whatever injury it may produce in the central nervous system, does not destroy the functional activity in the medullary centers.

The reader will recall that sight and hearing were present in the unanesthetized animals after exposure. In all animals the respiration, of course continued and in the anesthetized animals the corneal reflex was very active until just before death. These observations alone do not, however, exclude a functional disturbance in the medullary centers and particularly in the centers presiding over the heart-rate and vascular tone. The very marked depression of the arterial pressure at once suggested a loss of vasomotor control although the low venous pressure found coexistent with this low arterial pressure was contrary to such an interpretation.

The investigation of the cardio-motor, respiratory and vasomotor reflexes completely excluded such an explanation. If one distinguished a vasotonic and a vasoreflex mechanism as described by Porter (12) for the control of arterial tone, it it conceivable that vasoreflexes might persist after vasotonus has been lost. But here again the fact that the venous pressure fell coincidently with the fall in arterial pressure speaks against such an hypothesis. It is unfortunate that experimental conditions did not permit of a study of the peripheral resistance in these animals to determine the condition of arterial tone before and after exposure. Such a study would have helped to clarify this point.

Dogs 1 and 2, both in shock, gave such unmistakable evidence of the functional integrity of the medullary centers that the matter was not pursued further. The results are presented in table 7. It will be seen that the reactions to sensory nerve stimulation were unaffected. Before the stimuli were applied one vagus was cut. Peripheral vagus stimulation readily elicited cardiac inhibition, as might be expected from the reflex slowing of the heart indicated in the table. Peripheral sciatic nerve stimu-

lation, in the frog, likewise caused intensive muscular activity indicating again the absence of all peripheral neuro-muscular injury. The results

TABLE 7

Effect of sensory nerve stimulation upon pulse, respiration and arterial blood pressure

DOG NUMBER	NERVE	POSITION COIL	TIME	ARTERIAL PRESSURE		RESPIRATION		HEART RATE	
				Before	After stimulation	Before	After stimulation	Before	After stimulation
1	Saphenous	4	9:15	84	Slight fall	18	Accel.; dec. ampl.	150	
	Saphenous	4	10:25	36	No change	48	Slowed, dec. ampl.	156	
	Saphenous	4	11:35	46	Slight fall	60	Slowed, dec. ampl.	186	
	Saphneous	4	1:30	50	Slight fall	48	Slowed, dec. ampl.	198	
	Saphenous	4	2:30	50	Slight rise	48	Slowed, dec. ampl.	204	
	Saphenous	4	4:00	44	36	54	Slowed, slight dec. ampl.	204	
	Saphenous	4	4:40	42	Slight fall	54	Slightly slowed	198	
	Vagus centrally	10	9:15	84	78	18	Stopped	150	
	Vagus centrally	10	10:25	36	16	48	Stopped	156	
	Vagus centrally	10	11:35	46	26	60	Stopped	186	
	Vagus centrally	10	1:30	50	40	48	Stopped	198	
	Vagus centrally	10	2:30	50	40	48	Stopped	204	
	Vagus centrally	10	4:00	44	34	54	Stopped	204	
	Vagus centrally	10	4:40	42	32	54	Stopped	198	
	Vagus centrally	6	9:15	84	114	18	Stopped	150	Slowed
	Vagus centrally	6	10:25	36	76	48	Stopped	156	144
	Vagus centrally	6	11:35	46	91	60	Stopped	186	Slowed
	Vagus centrally	6	1:30	50	80	48	Stopped	198	Slowed
	Vagus centrally	6	2:30	50	80	48	Stopped	204	Slowed
	Vagus centrally	6	4:00	44	74	54	Stopped	204	Slowed
	Vagus centrally	6	4:40	42	72	54	Stopped	198	Slowed
2	Saphenous	12	1:00	54	Slight fall	22	Stopped	168	
	Vagus centrally	12	1:00	54	Slight fall	22	Stopped	168	Slowed
	Vagus centrally	6	1:00	54	94	22	Stopped	168	Slowed

Dog 1 exposed at 10:12.
Dog 2 exposed at 11:05.

given in the table indicate no differences whatsoever in the responses by the cardio-motor, vasomotor and respiratory centers before and after shock was established. That is to say, in respect to the vasomotor re-

sponses, the *absolute rise or fall* of arterial pressure induced by sensory nerve stimulation of different strengths was the same after the animal had been in shock for more than six hours as it was before exposure. Porter and Seelig and Lyon based their statements that the vasomotor mechanism is intact in shock upon the observation that the percentile rise or fall of arterial pressure was the same before and after the institution of shock. Here, however, we are dealing with absolute changes of pressure and it may be assumed that to raise the blood pressure 20 mm. Hg from an initial level of 45 mm. would require as great if not a greater reaction on the part of the vasomotor center as to raise it a similar amount from an initial level of 85 mm.

These nervous centers in the medulla at the base of the brain play an essential part in normal body function and their injury has long been considered capable of producing symptoms of shock. It is not surprising, therefore, that Mott (1) and others should have assumed injury to these centers in explaining concussion shock (commotio cerebri). Whether or not injury is done them, the present experiments conclusively demonstrate their functional integrity. We must therefore discard the hypothesis ascribing to them an important rôle in the etiology of concussion shock.

The medullary centers are not only not thrown out of function in concussion shock but they continue to function for an astonishingly long time after the low arterial blood pressure of shock has been established. Thus in dog 1 all the medullary reflexes elicited by sensory nerve stimulation before exposure were obtained with equal success after the arterial pressure had been at shock level for six hours and a half, at which time the observations were discontinued. Erlanger, Gesell and Gasser (14) found likewise that the functional capacity of the vasomotor mechanism persisted long after the arterial pressure had fallen to the shock level. It follows, therefore, that this vitally important center, at least, is not as susceptible to the injurious consequences of an impaired blood supply as has hitherto been assumed.

The results above described call to mind the observations of Githens and Meltzer (13), who found that repeated blows applied indirectly to the cranium in anesthetized dogs produced a condition of narcosis so that the animals after the anesthetic was removed were insensitive to pain for several hours. In the period various reflexes, including vasomotor reflexes, were obtained at times in an exaggerated form. No mention is made of the blood pressure level. At post-mortem, hemorrhages were found in the upper part of the cord accompanied by a laceration of the gray matter.

Condition of abdominal veins. The abdominal veins were examined in dogs 1, 2, 5 and 7, all of which showed the characteristic prompt fall in arterial pressure to a shock level following exposure. For convenience the results are tabulated (table 8). Dogs 1 and 2, inspected nine and four

hours respectively after exposure, showed narrow, inconspicuous and dark veins. Dogs 5 and 7, examined seven and thirty-five minutes respectively after exposure, showed full, enlarged and normal-colored veins.

In the recent literature on shock emphasis has been laid on the fact that the splanchnic veins are not engorged (15). Surgeons called upon to perform emergency laparotomies in war work reported the abdominal veins to be constricted and inconspicuous (16). Although these observations may properly be assumed to have been made upon cases which had suffered extensive hemorrhage and a number of hours after the causal factor was established, it is nevertheless probable that if a vasomotor collapse existed the veins would have been full and distended. This accords with the original observation of Porter (17) that the vasomotor

TABLE 8

Conditions and observations of abdominal veins

DOG NUMBER	ANESTHETIC	TIME EXPOSURE	TIME EXAMINED	APPEARANCE OF ABDOMINAL VEINS
1	Chloretone intraperitoneally	10:12 a.m.	8:10 p.m.	Inconspicuous, small, dark color
2	Morphia-ether	11:05 a.m.	3:15 p.m.	Inconspicuous, narrow. Along greater curvature stomach, definitely engorged
5	Chloretone	3:21 p,m.	3:28 p.m.	Very definitely engorged
7	Morphia-ether	2:30 p.m.	3:05 p.m.	Perfectly obviously engorged

mechanism is functional in deep shock. Seelig and Lyon (18), Morison and Hooker (19) and others have confirmed this observation. Quite recently Erlanger, Gesell and Gasser (20) have shown, as judged by temporary isolation and perfusion of vascular beds, that vasomotor tone is maintained in shock for a period of many hours and in the present experiments with concussion shock vasomotor reflexes persisted without any diminution for at least six hours and a half. These several observations completely refute the old theory that shock is due to a collapse of the vasomotor mechanism. In recent studies a reduction in the circulating plasma volume has been proposed to account for the circulatory failure in shock. Indeed, Erlanger, Gesell and Gasser (20), using the acacia method (21), have found a very considerable concentration of the blood. But in shock by air concussion which comes on with such suddenness, it is difficult to see how a loss of blood volume could be of prime importance. Transudation of blood plasma, furthermore, predicates a chemical injury to the vascular epithelium. It is extremely improbable that asphyxiation or the accumulation of toxic substances in these experimental animals could occur with such rapidity that sufficient plasma would transude from the

blood vessels to throw the arterial pressure to a shock level within ten minutes.

The appearance of the abdominal veins in animals shocked by air concussion if examined some hours after exposure, however, accords with the reported clinical observations. Thus in dog 1 the mesenteric veins were examined ten hours after exposure and a half-hour before death. At that time the veins were certainly not engorged: rather they were small, dark and inconspicuous. Essentially the same picture was found in dog 2 although in this animal the examination was made post-mortem, four hours after exposure. In the latter case note was made that the veins along the greater curvature of the stomach were engorged, in marked contrast to the veins of the intestinal mesentery. Probably no significance adheres to this detail.

The findings in these two dogs, examined late in shock, agree with the clinical observations and may be regarded as having been for some time in a condition conducive to the transudation of plasma. That is to say, the vascular epithelium had been exposed for some hours to a sluggish blood stream from which the products of metabolism, normal and abnormal, were not removed by the normal processes. In dogs 5 and 7, however, in which the abdominal veins were inspected shortly after exposure, the picture was entirely different. They gave perfectly clear evidence of venous engorgement.

In dog 5, examined seven minutes after exposure, the veins were very definitely engorged. This animal was anesthetized with an intraperitoneal injection of chloretone. This substance, although bland as judged by the absence of symptoms of local irritation, might be suspected of contributing to the dilated state of the veins since two hours after exposure the picture of engorgement was essentially unchanged. This explanation is not, however, tenable when it is recalled that dog 1, similarly anesthetized with chloretone, nine hours after exposure exhibited veins which were narrow and inconspicuous. Furthermore, dog 3 which was given three doses of chloretone intraperitoneally and was not exposed, did not show enlarged mesenteric veins. The possibility that chloretone might be a factor in contributing to the engorgement of the veins is entirely ruled out in the case of dog 7. This animal was anesthetized with morphia and ether and examined thirty-five minutes after exposure, light ether being required for the abdominal incision. The mesenteric veins were perfectly obviously engorged. Ten minutes later examination was made without ether; the evidence of engorgement was equally convincing. This second observation indicates that the use of ether for the first inspection did not invalidate the observation. Examination immediately after death, two hours and eight minutes after exposure, showed the veins no longer engorged. They were not, however, shrunken as in dog 1, but of normal size and very dark in color.

The results of these four experiments, therefore, indicate that shortly after concussion shock has been established, the abdominal veins are conspicuously enlarged so that they may be said to be engorged. Four hours or more after the establishment of shock the veins are no longer enlarged but shrunken, inconspicuous and dark in color.

The reader will recall that these animals also exhibited a low venous blood pressure. This finding means first that the vasomotor mechanism has not collapsed, and second that there is no impediment to the return flow of blood to the heart. The primary engorgement and subsequent shrinkage of the abdominal veins is then unrelated to any of the mechanical factors accepted as presiding over the venous pressure. There must have been an active loss of venous tone to give rise to the primary engorgement. The subsequent shrinkage of the veins may be readily explained on the assumption that a reduction in blood volume has occurred resulting in a decrease in their caliber. How this response on the part of the veins is mediated is a matter of speculation at the present time. The writer has been led to consider it related to some disturbance in function of the veno-pressor mechanism which he has shown to preside over the pressure in and consequently over the caliber of the mesenteric veins (22). The results indicate that the clinical reports made on the condition of the veins in shocked cases coming to operation tell only part of the vascular history in shock. They cannot be looked upon as excluding a venous engorgement early in the condition which was so conspicuous a feature in these experiments.

In connection with the condition of the abdominal veins attention should also be directed to the results on frogs. A number of frogs were examined shortly after exposure. On February 14 three frogs were exposed to about the same concussion. One, not examined, died within twenty-four hours. A second, examined five hours after exposure, showed no dilatation of the auricles or engorgement of the abdominal veins. A third, examined ten minutes after exposure, showed the ventricle beating but pale and empty. The auricles were widely distended and full of blood. Regular contractions were evident but the contained blood was not moved. The abdominal veins were conspicuously engorged. The same dilated condition of the veins was noted in frogs 1, 2, 3 and 4 exposed to trinitrotoluene on March 6.

The results on dogs and frogs presented above indicate that early in shock the veins of the splanchnic area are dilated and engorged with blood. In time this engorgement passes off and later still the veins may appear quite inconspicuous in size although they are clearly marked by their very dark color. In view of these findings, therefore, it may be questioned if the reports by surgeons of non-engorgement of the splanchnic veins represents a primary condition in shock unless it be associated with hemorrhage. The conditions observed in these animals suggest a primary paralysis of

the circulation accompanied by a pronounced engorgement of the veins. Since this engorgement disappears and is replaced in time by an actual decrease in the size of the veins, it is further suggested that subsequent to the initiation of shock metabolic disturbances intervene which result in a loss of blood plasma such as Erlanger and others have observed and that this loss of plasma leads to a return of vein volume to normal or even below normal.

The changes in the caliber of the veins above discussed is not associated directly with arterial or venous pressure changes. Early in shock the veins are dilated (engorged) at a time when both the arterial and venous pressures are low; late in shock the veins are shrunken without any definite change in the arterial or venous pressures having occurred. In the intervening period it may be accepted that transudation of plasma from the vascular bed has taken place. The decrease in vein volume late in shock is in all likelihood passive and associated with a decreased blood volume since there is not a corresponding rise in venous pressure. The dilated (engorged) veins seen early in shock, at a time when the venous pressure is low, cannot be regarded as a passive change and must therefore be due to an actual loss of tone.

Furthermore this change in the veins occurs while the vasomotor mechanism is functional and while, presumably, the arteriolar peripherinal resistance is normal or above normal, judging from Erlanger's results. And it occurs coincident with a pronounced fall in arterial blood pressure. We have no evidence upon which to base an explanation of this remarkable condition. But it may properly be asked if it arises in some primary injury to the venomotor mechanism. And if so could such an injury, involving the supply of blood to the heart entrain the sequence of events which lead up to the symptom complex of concussion shock?

Destruction of tympanic membrane. The tympanic membranes were examined post-mortem in five animals. In three (dogs 1, 2 and 7), in which shock was established, both membranes were extensively lacerated. In one (dog 16), which was not shocked, both membranes were destroyed and in the other (dog 8), likewise not shocked, the right membrane was intact while the left was lacerated, with blood in the external auditory canal.

These observations are not especially significant but so far as they go they indicate that an injury to the ear sufficient to lacerate the tympanic membrane is insufficient to cause shock.

Embolism. It was stated in connection with the description of the experiments with frogs that no evidence of gas embolism was to be found either in these animals or in dogs which were shocked. The hypothesis that such a factor is involved in cases of death due to the detonation of high explosive shells was based wholly upon theoretical grounds and, indeed,

in its original form the hypothesis conceived the gas embolism to be carbon dioxide (8). *A priori* such an explanation is improbable because of the suddenness and brevity of the period of high pressure. An analogy was drawn to caisson disease. The conditions which lead up to the latter malady are first a prolonged exposure to high atmospheric pressure, then a sudden release of the pressure so that the nitrogen gas which has been forced into solution under the high pressure leaves solution and aggregates into air emboli visible to the eye in the vascular bed (23). In no case in the present experiments (frogs 4, 7, 8, 10, 11, 12 and 13 and dogs 1, 2, 3, 7, 10, 17, 20 and 28) was there evidence of such embolism.

A second form of embolism as causative in shock has been suggested by Porter (24), namely, the liberation of fat in injuries to the long bones and subcutaneous tissues. Animals shocked by air concussion do not exhibit such injuries. Nevertheless the theory was given such prominence that a careful study of the present material was called for. Lieut. P. S. McKibben, in the Army Neuro-Surgical Laboratory, was good enough to undertake this investigation. This work has been published (25), hence only brief consideration of it is called for here. Porter predicated fat embolism in the lungs, blocking the blood flow to the left heart or in the capillaries of the vasomotor center, causing an anemia and consequent vasomotor collapse. Both of these conceptions fail of support in the experimental results in concussion shock already presented. A block in the pulmonary circuit would lead to a dilated right heart and subsequent high venous pressure, while a low venous pressure was actually observed. An anemia of the vasomotor center sufficient to cause the low arterial pressure noted in the shocked animals would abolish vasomotor reflexes while these reflexes were readily elicited.

McKibben did indeed find intravascular fat in the cerebral vessels of dogs shocked by air concussion, but he also found an equal amount in the same vessels in dogs killed by ether inhalation and by bleeding. It follows, therefore, that the intravascular fat present in the shocked animals is of no significance as a causative factor in the condition.

Pathology. It is unfortunate that, although this report has been delayed for the purpose, the results of Professor Miller's study of the physical conditions surrounding the experiments as well as a consideration of the histological appearances of the body tissues can not be presented.

While the writer does not feel competent to discuss the microscopic pathology of the material preserved in these experiments, it is advisable to refer to some notes made by himself and others bearing particularly upon the question of hemorrhages in the central nervous system.

After the animals died or were sacrificed, the tissues and particularly the brain and upper cord were removed and placed in formalin. The protocols of the experiments carry some of the notes on the pathological

conditions found at autopsy. Although the brain and cord surfaces were carefully searched, no evidence of superficial hemorrhage was discovered. The veins frequently stood out conspicuously but there was no evidence of rupture. This observation was confirmed by a later and more careful study. Thus in dogs 1, 2, 7, 10 and 28 the notes at hand indicate no evidence of vascular injury in the central nervous system. In the case of dog 7, microscopic examination was made of sections from the cortex, cerebellum, medulla and cervical cord. These sections showed no hemorrhages within the substance of the brain or cord or their membranes. Similarly in dog 28 sections of the cortex, pons, cerebellum, medulla and optic chiasma revealed no microscopic hemorrhages. In dogs 1, 2 and 10 gross examination showed in each case an abnormal congestion of the veins over the brain. In one case the filled veins were in the pia mater (cerebral veins); in one case they were in the dura (cranial veins); and in the third case some veins were filled in both the pia and dura. In many places areas of what appeared to the naked eye as confluent or petechial hemorrhages (especially in the dura) proved, on examination with a high power binocular microscope, to be only a wide dilatation of the fine network of the smallest veins.

These observations clearly indicate the absence of hemorrhages, petechial or gross, in the nervous tissues after concussion shock. On the other hand the cytological appearance of these tissues (dog 28) was not entirely normal; the Nissl substance was scattered with a shrinkage and vacuolarization of the cytoplasm. The writer is not competent to interpret these apparent abnormalities.

As indicated in the first footnote and in the protocols, a considerable amount of the tissue was preserved for histological investigation. As planned, this material was placed in the hands of a competent neurologist, who has failed, doubtless because of wholly uncontrollable circumstances, to make the promised study. The visceral tissues all exhibited a normal histological picture except the lungs, which as already indicated, showed extensive areas of hemorrhage. The author cannot go beyond the above brief reference to condition of the nervous tissue. This is unfortunate because the experimental results indicate by exclusion, a primary lesion in the nervous and particularly in the higher nervous tissues which initiates the symptom complex of concussion shock.

Literature on concussion shock. The war stimulated renewed interest and an immense amount of work in the study of shock. These investigations may be divided into two categories; those dealing with secondary shock, a condition of low arterial blood pressure and collapse following extensive wounds with hemorrhage and exposure, and those dealing with primary shock, a condition in its outward manifestations analogous to secondary shock but which is by contrast developed suddenly. The former may not develop for hours while the latter occurs almost

instantly as a result of the causal event. The latter condition—primary shock—is further characterized by the absence of hemorrhage and other external evidences of injury. This is concussion shock, always associated clinically with exposure to the detonation of high-explosive shells. It is primary or concussion shock with which this paper deals. The literature on the subject is limited because concussion shock was practically unknown prior to the war.

In the first place reference may be made to case reports in which particular injuries have been found unrelated to a condition of shock. Thus Gatti (26) presents a case of extremely acute praralysis with atrophy following close on the explosion of a large bomb without actual contact. The condition was similar to that seen in anterior poliomyelitis and the assumption is made that the air concussion injured the anterior portion of the spinal cord. Similarly Cramer (27) reports the case of a young soldier who presented the signs of a severe aortic insufficiency after being thrown several feet into the air by the explosion of a bomb. This patient had previously been healthy and had not experienced the diseases which predispose to heart disease.

Other cases of a like character are on record indicating that air concussion may establish specific lesions irrespective of the effect upon the body as a whole. This we should expect. The variability of the physical conditions produced by the detonation referred to in the body of this paper, together with variations in individual resistance, posture, etc., might combine to produce injuries of a most diverse sort. It is not improbable that many of the cases which exhibited a bizarre clinical picture and which were therefore classified and treated as psychoses belong in this category. Mott (28) in his Lettsomian Lectures and in a subsequent communication (29) presents the pathological findings in the brains of several men reported as having died of primary shock. One of these cases may be detailed as characteristic of all.

Clinical notes. Admitted with diagnosis of shell shock. Purple bruises on arm and leg of right side. Stertorous, unconscious, and during the night before death, constant fits. Lived thirty hours in hospital.

Post mortem. There were two hematomata in right lung but no other visceral injury. No hemorrhage of the scalp and no fractures of the skull. Some slight subpial hemorrhage of the right hemisphere. Fornix destroyed and full of hemorrhages; hemorrhages also seen in corpus callosum. Hemorrhages in both optic thalami; cerebrospinal fluid tinged with blood. Men admitted with him said he had been buried by a shell. There was no CO in his blood, and the bruising was purple.

Microscopic examination. Multiple punctate hemorrhages are seen; hyaline thrombosis of capillaries, arteries and venules; perivascular sheaths contain blood. Marked evidence of inflammatory stasis. Some of the small veins are filled with blood corpuscles, one half of which are polymorphonuclear leucocytes, and in the perivascular sheath and tissues around are large numbers of polymorph leucocytes.

Two points are of interest in this typical case. First, the hematomata present in the lung. It will be recalled that these areas were uniformly present in my animals but that they bore no relationship to shock. Second, the extreme vascular injury to the brain. My animals showed blood vessels distended and engorged both on the surface and in the depths of the brain but in no case were petechial or other hemorrhages found. It is possible that the duration of shock before death ensues is a determining factor in the appearance of such hemorrhages. Their occurrence in any case is not necessarily a primary factor in shock.

Mott lays much emphasis on the similarity of the pathological picture in the central nervous system of cases dead of shock and of carbon monoxide poisoning. His description of the vascular lesions and his conclusions therefrom make it evident that two such brains can scarcely be distinguished. He is therefore strongly inclined to the view that carbon monoxide poisoning must play a large part in the fatal outcome of aerial concussion. The victim, stunned by the concussion, often in a dug-out or trench, lies on the ground breathing the gas in considerable concentration. Mott states that the failure to recognize CO in the blood post-mortem is insufficient evidence for discarding his hypothesis.

In a later paper Mott (30) presents observations which somewhat modify his opinion of concussion shock as transcribed above. Here he describes two cases of death from shock, without visible injury and without punctate hemorrhages indicative of gas poisoning.

The first case is complicated by a protracted period of nervousness (six months) prior to the event which presumably caused death. The man was in an area which was subjected to an intense bombardment for many hours. A shell burst within ten feet after which he developed tremors and general depression. These symptoms soon became accentuated: coarse tremors of the limbs, crying, inability to walk and to answer questions. A few hours later he was in acute mania. Morphia and chloroform were used to quiet him. He slept through the night and woke up apparently well. Within a short time he was found dead, approximately twenty-four hours after the onset of acute symptoms.

Post mortem. A number of minute punctiform hemorrhages were found on the surface of the brain. The whole brain was soft but not markedly edematous. Histologically there was "a generalized early chromatolytic change in the cells of the central nervous system." Subpial microscopic hemorrhages were found "almost everywhere." The veins of the white matter of the corpus callosum, internal capsule, pons and medulla were congested, with hemorrhages into the sheaths and occasionally extravasations of blood into the adjacent tissues.

The second case is more typical of concussion shock in that the man, with no previous history of nervousness, suddenly became unconscious with

stertorous breathing. He was exposed to the detonation of some boxes of cordite cartridges which were exploded by a shell falling upon them. He remained unconscious until death some twelve hours later. Superficial hemorrhages were found post mortem at various points on the surface of the brain.

The outstanding features of this case are the sudden onset associated with air concussion, and the short period which elapsed before death. Post mortem "the cortical arterial and capillary vessels were empty, the perivascular sheaths were dilated and filled presumably with cerebrospinal fluid. The cortical neurones are swollen up,—the nuclei are large and clear; the basophil substance is diminished in amount, a condition very like that observed in cells of the cortex of an animal in which experimental anemia has been effected." There was an extensive hemorrhage on the under-surface of the orbital lobe.

On the basis of these two cases Mott adopts the view that the compression and decompression of the atmosphere incident to the detonation of an explosive act to produce "vascular disturbances in the central nervous system, causing arterio-capillary anemia and venous congestion and rupture of delicate walled vessels with microscopic hemorrhages." It may be inferred that these vascular changes are regarded by him as antecedent to the cellular changes and represent therefore the primary derangement in concussion shock.

The results reported in the present paper do not accord with this view. No hemorrhages, gross or microscopic, were found in the central nervous system in any of the experimental animals, athough some of them were so severely shocked that death occurred within two hours or less. We cannot, therefore, regard vascular lesions in the brain as an essential feature of concussion shock. (See dogs 1, 2, 7, 10 and 28.)

Carver and Dinsley (2) report experiments on fish, rats and mice. The fish were exposed by detonating high explosive in the water in which they were contained; the rats and mice were exposed in small cages. Some of the fish were killed outright. Others were stunned; the bodies were rigid and floated vertically in the water. After a half-hour swimming movements began. These first movements occurred with the ventral surface uppermost, i.e., before the power of equilibrium was regained. The majority of fish which thus attained the power to swim gradually became more and more active so that, in about twelve hours after the detonation, those which had not entirely succumbed were to all appearances completely normal. It may be remarked in passing that fish, however injured, present very similar symptoms so that one is not inclined to regard the behavior described as of special significance in shock.

Post-mortem examination revealed "hemorrhages in the upper region of the spinal cord and medulla, congestion of the fore parts of the brain, and

a number of hemorrhagic points in the mid-brain and basal gangliar region. The gills and other viscera were congested and often showed areas of hemorrhage. Fish taken at random immediately after the detonation revealed either varying degrees of the above-mentioned lesions, or by macroscopic examination nothing abnormal was discovered."

The rats and mice, which escaped direct trauma, were thrown into a state of stupor or transitory loss of consciousness depending upon the distance separating them from the center of the detonation. "During the course of recovery twitching movements of the limbs were often present, and then the animal, if stimulated by appropriate physical agents, showed massive and exaggerated reflex reactions. For example, the application of a pin-prick to one hind limb might at this stage be followed by a bilateral flexion of the hinder extremities, or even by a generalized convulsion." They noted also that a "rapid fatigue of the response on repeated stimulation was a noticeable feature."

The behavior and responses on the part of these animals is quite similar to those described in the present paper for frogs. Carver and Dinsley state that such animals when sacrificed showed no gross macroscopic evidence of internal injury. It is inferred that they refer to the central nervous system since it is not credible that the lungs were intact. Animals however, which were more severely exposed showed hemorrhages from the ears, nose and mouth and when sacrificed exhibited "varying amounts and degrees of internal hemorrhage in the viscera and central nervous system; the alveoli of the lungs were almost always found to be to a certain extent ruptured."

The writers were unable to obtain microscopic sections of their material but they refer to a condition of "general capillary engorgement—especially noticeable in the central nervous system and meninges." As the result of their work the authors incline strongly to the belief that there is a physical basis for many of the war psychoses etiologically dating from exposure to a detonating shell.

On the pathological side of the subject, the paper by Mairet and Durante (2) is the most important to which the writer has had access.

Mairet and Durante have published several papers on concussion shock, the last of which (2) presents the conclusions and opinions based on a considerable amount of experimental work. They exposed rabbits to the detonation of high explosive under various conditions and made a particular study of the pathological lesions thereby produced. The symptomatic behavior of the animals was incidentally noted. This naturally varied with the amount of the explosive used and the distance of the animals from the charge. Those lightly affected appeared slightly stunned with a rapid respiration, while of those severely affected, some were greatly depressed and died within the hour and others showed a stage of excitement and

subsequent recovery. The animals which did not die in the first twenty-four hours recovered without the subsequent development of symptoms indicative of central nervous system injury.

The authors divide the post-mortem findings into early and late lesions. By early lesions are meant the conditions found in animals sacrificed by bleeding shortly after exposure. The viscera were normal except for the lungs. These organs were spotted with hemorrhagic areas which sometimes became confluent covering half or two-thirds of a lobe. This condition has been recognized by numerous observers. In the present experiments it was the sole gross lesion universally found. On the surface of the cord in all cases they found varying amounts of blood suffusion irregularly distributed with small blood clots adhering to the nerve roots. The brain appeared normal or showed petechial hemorrhages at various points on the surface.

Microscopically the nerve roots showed a hemorrhagic condition in the interstitial tissue. The nerve bundles themselves were unharmed as were also the ganglia. These hemorrhagic areas were especially conspicuous in the free floating portions of the roots, a fact which leads the authors to the conception that the air vibrations transmitted to these loose-lying structures are responsible for this characteristic lesion. From this they reason that the clinical symptoms of concussion shock, many of which suggest spinal cord disturbance, are in part dependent upon hemorrhages in the spinal roots. While this deduction may be correct, it is not out of place to inquire why none of the animals which were allowed to live showed locomotor difficulties.

The cord similarly showed minute hemorrhagic areas. The motor and sensory cells appeared normal. The nerve trunks and nerve fibers were however affected. The myelin sheaths were broken and the axis-cylinders were irregular in outline, swollen, coalescing or even lacking in places. Somewhat similar conditions of hemorrhage appeared in the brain substance but here there was no marked indication of tissue injury.

The animals used for the study of late lesions were sacrificed from five to eight months after exposure. In the intervening period they had seemed entirely normal without the development of paralysis, atrophy, anesthesia, etc. No macroscopic lesions were evident in the brain or cord. We are not here especially interested in the microscopical findings of this series of animals. It may be said, however, that the authors recognized lesions in the brain which might have originated in microscopic infarcts or submeningeal hemorrhages. The motor cells of the cortex were atrophied and vacuolated and there was an appearance of columnization of the cells ascribed to the destruction of parallel radiating capillaries.

The above abstract of the findings of Mairet and Durante will serve to bring out the points which they emphasize, namely, the presence of minute

scattered hemorrhages in the central nervous system and the absence of specific nerve cell injury as an acute process. They conceive of the areas of microscopic hemorrhage as leading to an attrition of various nerve cells most conspicuous in the motor cortex but present presumably throughout the central nervous system. By inference we are led to conclude that the varied symptomatology of concussion shock in clinical cases is to be ascribed to the development of such lesions. Such a conclusion does not find support, however, in their experimental material since animals allowed to live for months after exposure did not develop any symptoms suggestive of such lesions.

The authors emphasize the fact that the hemorrhagic lesions are especially conspicuous in parts of the nervous system, i.e., the nerve roots, which are relatively unsupported by neighboring tissues and are therefore more liable to injury by oscillatory movements. Upon this observation they develop a theory that concussion shock is due to the vibrations set up in the atmosphere which, transmitted to the organism, act injuriously by violently shaking the unprotected tissues. The bearing of this theory on the present experiments has already been discussed and it is sufficient to repeat that it seems inadequate to explain all the results obtained.

It should be pointed out here also that the material from the present experiments failed to show any evidence of hemorrhage. Neither macroscopically nor microscopically was extravascular blood discovered either on the surface or in the depths of the nervous tissue. The conclusion is therefore inevitable that the development of petechial hemorrhages is not essential for the production of concussion shock.

SUMMARY AND CONCLUSIONS

Frogs, rabbits, cats and dogs were exposed to the atmospheric disturbances (air concussion) produced by the firing of large guns, a procedure which established a condition of "primary shock."

In this work a considerable and sudden drop in arterial blood pressure is taken as the criterion of shock.

Reasons are advanced to show that the effect is due to the duration of the phase of high atmospheric pressure rather than to the height of the atmospheric pressure as such or to the tonal vibrations of the atmosphere.

The major effort was directed to a study of the physiological phenomena associated with the drop in arterial pressure, the outcome being that shock by air concussion is a condition distinct from other forms of shock hitherto considered. The data gathered may be of value, however, in elucidating other forms of shock, particularly with respect to the sequence of events leading up to the condition.

The experiments are classified in the paper and the results and conclusions drawn from them are presented below.

In the *preliminary experiments with unanesthetized animals*, a form of shock was clearly developed. Immediately after exposure the animals showed marked lethargy and fatigue. Upon this condition, akin to exhaustion, was later superimposed respiratory distress, which increased in severity and was probably the immediate cause of death. This respiratory distress was presumably due largely if not wholly to hemorrhagic lesions in the lungs. The pulmonary injury, however, was not related to the prominent condition of exhaustion.

The *experiments with frogs*, which were exposed without anesthesia, indicated *a*, that the positive and not the negative phase of atmospheric pressure, as has been suggested, is the causal factor since when rupture of the lungs occurred air was found within the body cavity; *b*, that there is no experimental support for the hypothesis that air emboli, formed by the negative pressure phase, are the cause of death since no air emboli were ever found in the vascular system; and *c*, that there is an engorgement of the great veins early in the condition, an engorgement which subsides in the course of time.

The *experiments with high explosive* (trinitrotoluene) emphasize the fact that the injury done the lungs in these and other experiments is not causal to shock since exposure to high explosive in no case produced typical shock and yet the pulmonary involvement was of extremest grade. They indicate further that extensive laceration and destruction of lung tissue does not release a toxic substance, i.e., histamine, into the circulation in sufficient quantities to produce a physiological reaction.

The *experiments with the gun blast*, using anesthetized dogs, gave the most satisfactory results and were utilized for an analysis of the physiological aspects of the shock thereby produced. Exposure of animals from ten to twenty feet in front of the ten- and twelve-inch rifles yielding a concussion pressure of eighteen or nineteen atmospheres per square inch usually produced primary shock. This condition is essentially instantaneous in onset exhibited by a sudden marked fall in arterial blood pressure.

Coincident with the low arterial blood pressure, the venous pressure is likewise lowered indicating a loss of tone in the veins. This indication of an independent loss of venous tone is substantiated by the concomitant venous engorgement noted early in the condition of shock.

There is no evidence that the functional capacity of the heart was affected and the valves were uninjured.

Immediately after shock is established the CO_2 capacity of the blood is normal. With the persistence of the low arterial pressure characteristic of the condition, the CO_2 capacity is materially decreased. The "acidosis" hypothesis cannot therefore apply to concussion shock.

The blood is not hemolyzed in concussion shock.

Although the determinations were quite unsatisfactory, the deduction is made that blood catalase is not affected.

The functional activity of the medullary centers was wholly normal. Vasomotor, cardiomotor and respiratory reflexes were readily elicited. Other reflexes, i.e., spinal, eyelid and swallowing, were similarly present.

The abdominal veins were markedly enlarged early in shock. This enlargement was accompanied by a low venous pressure so that it is not strictly correct to say that they were engorged although their appearance gave such an impression. In the course of two hours or more this enlargement gave place to an actual decrease in size due, it is presumed, to a transudation of blood plasma. Since the venous pressure was subnormal, it is concluded that enlargement of the veins was due to an active loss of tone in their walls. The observation accords with clinical experience that the abdominal veins are not engorged late in shock. The fact, however, that in these experiments the veins were enlarged early in shock suggests that the clinical reports on the condition of the veins cannot be taken without reservation as indicating no participation of the venous bed in the circulatory failure of shock.

The concussion pressure adequate to produce shock results in extensive laceration of the tympanic membrane of the ears.

No evidence of embolism (air) was found in any of the experiments.

No evidence of hemorrhage, gross or petechial, has been found in the nervous tissues thus far studied.

Dog 1. February 9, 1918. Male, weight 26 pounds.

7:30 a.m.	2.4 grams chloretone in oil intraperitoneally. Narcotized promptly, lies by warm radiator.
7:55	Pulse 126, respiration 11 per minute.
9:10	Operation completed.
9:15	Arterial pressure 84 mm. Hg, pulse 150, respiration 18. Saphenous stimulation, coil 4, slight drop in B.P., respiration accelerated with decreased amplitude.
	Vagus peripherally, coil 10, inhibited heart.
	Vagus centrally, coil 10, lowered B.P. 6 mm. Hg, respiration inhibited.
	Vagus centrally, coil 6, raised B.P. 30 mm. Hg, respiration inhibited, pulse slowed.
	Temperature 36°C.
	Venous pressure: Ext. jug. 2.5 cm., Fem. 6.5 cm. H_2O.
10:12	Exposed 18 feet in front of 10-inch rifle.
10:22	Returned to laboratory. Before exposure the animal showed signs of weakened anesthesia; after exposure it was completely quiescent.
10:25	Arterial pressure 36 mm. Hg, pulse 156, respiration 48. Saphenous stimulation, coil 4, no change in B.P., respiration slowed with decreased amplitude.
	Vagus centrally, coil 10, lowered B.P. 20 mm. Hg, respiration inhibited.
	Vagus centrally, coil 6, raised B.P. 40 mm. Hg, respiration inhibited, pulse slowed 12 per min.

10:25 a.m. Temperature 34°C.
Venous pressure: Ext. jug. −6.0 cm., Fem. 2.5 cm. H_2O.
11:35 Arterial pressure 46 mm. Hg, pulse 186, respiration 60. Saphenous stimulation, coil 4, slight drop in B.P., respiration slowed with decreased amplitude.
Vagus peripherally, coil 10, inhibited heart.
Vagus centrally, coil 10, lowered B.P. 20 mm. Hg, respiration inhibited.
Vagus centrally, coil 6, raised B.P. 45 mm. Hg, respiration inhibited, pulse slowed.
Temperature 34°C.
Venous pressure: Ext. jug. −6.5 cm., Fem. −1.0 cm. H_2O.
1:30 p.m. Arterial pressure 50 mm. Hg, pulse 198, respiration 48. Saphenous stimulation, coil 4, slight drop in B.P., respiration slowed with decreased amplitude.
Vagus peripherally, coil 10, inhibited heart.
Vagus centrally, coil 10, lowered B.P. 10 mm. Hg, respiration inhibited, pulse unchanged.
Vagus centrally, coil 6, raised B.P. 30 mm. Hg, respiration inhibited, pulse slowed.
Temperature 34.5°C.
Venous pressure: Ext. jug. −6.25 cm., Fem. 1.0 cm.
2:30 Arterial pressure 50 mm. Hg, pulse 204, respiration 48. Saphenous stimulation, coil 4, slight rise in B.P., respiration slowed with decreased amplitude.
Vagus peripherally, coil 10, inhibited heart.
Vagus centrally, coil 10, lowered B.P. 10 mm. Hg, respiration inhibited.
Vagus centrally, coil 6, raised B.P. 30 mm. Hg, respiration inhibited, pulse slowed.
Temperature 35°C.
Venous pressure: Ext. jug. −5.75 cm., Fem. 3.75 cm. H_2O.
4:00 Arterial pressure 44 mm. Hg, pulse 204, respiration 54. Saphenous stimulation, coil 4, lowered B.P. 8 mm. Hg, slight slowing of respiration with very slight decrease in amplitude.
Vagus peripherally, coil 10, pulse slowed.
Vagus centrally, coil 10, lowered B.P. 10 mm. Hg, respiration inhibited.
Vagus centrally, coil 6, raised B.P. 30 mm. Hg, respiration inhibited, pulse slowed.
Temperature 35°C.
Venous pressure: Ext. jug. −6.0 cm., Fem. 2.25 cm. H_2O.
4:40 Arterial pressure 42 mm. Hg, pulse 198, respiration 54.
Saphenous stimulation, coil 4, slight drop in B.P., slight slowing of respiration with no change in amplitude.
Vagus peripherally, coil 10, inhibited heart.
Vagus centrally, coil 10, lowered B.P. 10 mm. Hg, respiration inhibited.
Vagus centrally, coil 6, raised B.P. 30 mm. Hg, respiration inhibited, pulse slowed.
Temperature 35.5°C.
Venous pressure: Ext. jug. −1.5 cm. H_2O. Clot in femoral veins.
7:45 Arterial pressure 40 mm. Hg, pulse 228, respiration 54.
Temperature 37.25°C.
Corneal reflex very active.

8:10 p.m. Abdomen opened. Mesenteric veins certainly not engorged. Rather they are inconspicuous, small and dark in color.
8:40 Venous pressure, portal via splenic vein, 12.75 cm.
8:43 Respiration stopped. Eye lid reflex absent. Dead. Heart continued beating. Blood-stained fluid exuded from nose.
9:00 Post mortem. Abdomen clear. Lungs showed widely scattered solid, bright red areas particularly at base and back. Injected with formalin. Brain and upper cord preserved in formalin. Both tympanic membranes extensively ruptured.

Note: When not under observation animal was placed near warm radiator.

Dog 2. February 11, 1918. Male, weight about 25 pounds.

7:45 a.m. One grain morphia hypodermically.
8:30 One-half grain morphia hypodermically.
9:50 Operation completed under light ether.
Corneal reflex sharp.
10:00 Arterial pressure 150 mm. Hg, pulse 60, respiration 12.
Venous pressure: Ext. jug. 4.5 cm., Fem. 15.5 cm. H_2O.
Catalase 165 cc. at 23°C., 766 mm. Hg.
Temperature 33°C.
11:05 Exposed 18 feet in front of 10-inch rifle.
Femoral vein ligature slipped; bled perhaps 100 cc.
11:35 Arterial pressure 50 mm. Hg, pulse 180, respiration 16.
Venous pressure: Ext. jug. −3.0 cm., Fem. 5.75 cm. H_2O.
Catalase 128 cc.
Corneal reflex sharp.
Temperature 30.5°C.
1:00 p.m. Arterial pressure 54 mm. Hg, pulse 168, respiration 22.
Saphenous stimulation, coil 12, slight drop in B.P., respiration inhibited.
Vagus centrally, coil 12, slight drop in B.P., respiration inhibited, pulse slowed.
Vagus centrally, coil 6, raised B.P. 40 mm. Hg, respiration inhibited, pulse slowed.
Venous pressure: Ext. jug. −3.0 cm., Fem. 5.75 cm. H_2O.
Catalase 95 cc.
Corneal reflex sharp. Spontaneous body movements. Occasional sighing respiration.
2:05 Arterial pressure 48 mm. Hg, pulse 172, respiration 20.
Venous pressure: Ext. jug. −2.0 cm., Fem. 0.5 cm. H_2O.
Corneal reflex sharp. Animal swallows.
2:45 Arterial pressure 42 mm. Hg, pulse 129, respiration 18.
Venous pressure: Ext. jug. −3.0 cm., Fem. 5.5 cm. H_2O.
3:07 Dog dead. Heart beats felt after cessation of respiration. Blood-stained fluid exuded from nose.
3:15 Post mortem. Mesenteric veins not swollen—quite inconspicuous. Veins along greater curvature of stomach definitely engorged. Considerable pericardial fluid—colorless and clear. No fluid in chest or abdomen. Injected with formalin. Brain and upper cord preserved in formalin.
Intestines and spleen grayish blue. Posterior stomach wall (outside) shows two hemorrhagic areas, 40 and 60 mm. diameter. Mucosa

beneath entirely normal. Lungs diffusely reddened. Areas smaller than in dog 1 but more numerous.
Both tympanic membranes extensively ruptured.

Dog 3. Control. February 12, 1918. Female, weight 20 pounds.

7:45 a.m. 1.8 grams chloretone in oil intraperitoneally.
9:45 Operation completed with ether occasionally.
10:00 Abdomen opened to observe mesenteric veins. Normal in appearance.
10:10 Arterial pressure 90 mm. Hg, pulse 144, respiration 32.
Venous pressure: Ext. jug., 1.0 cm., Fem. 4.25 cm. H_2O.
Catalase 105 cc. at 22°C., 757 mm. Hg.
Corneal reflex definite.
Temperature 35.75°C.
10:58 Catalase 118 cc.
11:15 0.4 gram chloretone.
11:50 0.4 gram chloretone.
1:30 p.m. Tracheotomy and continuous ether.
1:35 Arterial pressure 98 mm. Hg, pulse 208, respiration 100.
Venous pressure: Ext. jug. 0.75 cm., Fem. 2.75 cm. H_2O.
Catalase 121 cc. at 34°C.
2:45 Arterial pressure 68 mm. Hg, pulse 220, respiration 92.
Venous pressure: Ext. jug. 0.5 cm., Fem. 3.5 cm. H_2O.
3:00 Chloroformed. Post mortem. Mesenteric veins normal in appearance. Large round hole with thickened edge in pericardium. No pericardial fluid. Lungs looked about as shocked lungs but seemed dryer.
No specimens preserved.

Dog 4. Control. February 13, 1918. Male, weight about 20 pounds.

7:15 a.m. One grain morphia hypodermically.
7:45 One-half grain morphia.
8:15 Operation completed under ether.
8:17 Catalase 115 cc. at 25°C., 758 mm. Hg.
8:45 Arterial pressure 118 mm. Hg, pulse 80, respiration 64.
Venous pressure: Ext. jug. 1.5 cm., Fem. 9.5 cm., H_2O.
9:00 Abdomen opened to observe mesenteric veins. Normal in appearance.
9:08 Catalase 132 cc. at 25.5°C.
10:45 One grain morphia.
11:15 Catalase 133 cc. at 27°C.
11:30 Arterial pressure 116 mm. Hg, pulse 126.
Venous pressure: Ext. jug. 0.25 cm., Fem. 7.0 cm. H_2O.
11.45 Mesenteric veins slightly darker, otherwise normal in appearance.
11.50 Chloroform intravenously.
Post mortem. Lungs showed much the same red areas as seen in shocked animals but not so diffusely scattered. Injected with formalin. Brain and upper cord preserved in formalin.

Dog 5. February 15, 1918. Female, weight 22 pounds.

1:55 p.m. 2.5 grams chloretone in oil intraperitoneally.
2:40 Arterial pressure 70 mm. Hg, pulse 123, respiration 18.
Venous pressure: Ext. jug. 1.0 cm., Fem. 2.75 cm. H_2O.
Catalase 16.7 cc. at 24.5°C., 755 mm. Hg.

3:21 p.m. Exposed 18 feet in front of 10-inch rifle.
3:28 Catalase 9.8 cc. at 23°C.
Abdomen opened. Veins very definitely engorged.
3:35 Arterial pressure 46 mm. Hg, pulse 102, respiration 72.
Venous pressure: Ext. jug. −0.5 cm., Fem. 4.0 cm. H_2O.
4:30 Catalase 16 cc. at 23.75°C.
Arterial pressure 40 mm. Hg, pulse 104, respiration 104.
Venous pressure: Ext. jug. −1.0 cm., Fem. 3.0 cm. H_2O.
5:30 Arterial pressure 42 mm. Hg, pulse 156, respiration 144.
Venous pressure: Ext. jug. 0.5 cm., Fem. 3.5 cm. H_2O.
Condition of mesenteric veins same as at first examination.
5:35 Animal sacrificed. No post mortem. No specimens preserved

Dog 6. February 16, 1918. Male, weight about 28 pounds.

7:00 a.m. One grain morphia hypodermically.
8:15 Operation completed under light ether.
9:40 One-half grain morphia.
11:20 Arterial pressure 110 mm. Hg, pulse 123, respiration 20.
Venous pressure: Ext. jug. 0.0 cm., Fem. 4.5 cm. H_2O.
Catalase 145 cc. at 24°C., 758 mm. Hg.
11:49 Exposed 100 feet in front of 10-inch rifle.
11:56 Catalase 172 cc. at 24.5°C.
12:00 Arterial pressure 108 mm. Hg, pulse 136, respiration 12.
Venous pressure: Ext. jug. 1.0 cm., Fem. 6.0 cm. H_2O.
12:55 Arterial pressure 106 mm. Hg, pulse 128, respiration 20.
Venous pressure: Ext. jug. 0.5 cm., Fem. 4.5 cm. H_2O.
Catalase 161 cc. at 23.5°C.
1:44 Exposed 15 feet in front of 10-inch rifle, firing small charge.
2:00 Catalase 165 cc. at 22°C.
Arterial pressure 112 mm. Hg, pulse 228, respiration 20.
Venous pressure: Ext. jug., −1.0 cm., Fem. 4.0 cm. H_2O.
2:07 Arterial pressure 106 mm. Hg, pulse 222, respiration 36.
2:10 Abdomen opened. Veins seem enlarged but not markedly so.
2:40 Arterial pressure 90 mm. Hg, pulse 235.
Venous pressure: Ext. jug. −0.25, Fem. 4.5 cm. H_2O.
Sacrificed. No post mortem.

Dog 7. February 27, 1918. Female, large.

12:00 m. One grain morphia hypodermically.
1:20 p.m. Operation completed under light ether.
1:30 Arterial pressure 136 mm. Hg, pulse 99, respiration 21.
Venous pressure: Ext. jug. 3.5 cm., "effective" 15.25 cm. H_2O.
Alkaline reserve 69.1 per cent.
Catalase 24 cc. at 23.25°C., 766 mm. Hg.
2:30 Exposed 10 feet in front of 12-inch rifle.
2:32 Back in laboratory. Respiration very rapid and deep.
2:35 Alkaline reserve 69.1 per cent.
Catalase 13 cc. at 22.5°C.
Arterial pressure 66 mm. Hg, pulse 114, respiration 70. Occasional sighing respiration.
Venous pressure: Ext. jug. 5.75 cm., "effective" 4.75 cm. H_2O.

3:05 p.m. Abdomen opened under light ether. Veins perfectly obviously engorged.
3:15 Arterial pressure 90 mm. Hg, pulse 163, respiration 63.
Mesenteric veins again examined under ether—still engorged. Grunting respiration.
Venous pressure: Ext. jug. 5.5 cm., "effective" 2.0 cm. H_2O.
Corneal reflex sharp.
Catalase 19 cc. at 22°C.
4:00 Bleeding at mouth. Occasional grunting respiration.
Arterial pressure 85 mm. Hg, pulse 168, respiration 45.
4:15 Alkaline reserve 43.8 per cent.
Blood dripping from mouth.
4:30 Arterial pressure 70 mm. Hg, pulse 120, respiration 21.
Venous pressure: Ext. jug. 4.5 cm., "effective" 6.0 cm. H_2O.
4:37 Arterial pressure 20 mm. Hg, pulse 39, respiration 1.
4:38 Dead.
Post mortem. Abdomen clear. Mesenteric veins not engorged. Heart normal, small amount of blood-tinged pericardial fluid. Lungs show scattered solid-red areas of small size. Lower lobes have massed solid-red areas. Similar solid-red areas well developed at lung roots, 3 to 4 cm. in diameter. Blood-stained froth in trachea. An apparent blood clot in midline of dorsal surface of brain was not disturbed.
Injected with formalin. Brain and upper cord preserved in formalin.
Both tympanic membranes ruptured.

Dog 8. February 28, 1918. Male, large.

2:30 p.m. One grain morphia hypodermically.
3:27 Operation completed under light ether.
3:35 Arterial pressure 138 mm. Hg, pulse 150, respiration 72.
Venous pressure: Ext. jug. 4.5 cm., "effective" 5.0 cm., H_2O.
Alkaline reserve 39.5 per cent.
Catalase 9 cc. at 23°C., 767 mm. Hg.
3:50 One-half grain morphia hypodermically.
4:46 Exposed 5 feet in front of 12-inch rifle.
4:54 Alkaline reserve 46.2 per cent.
Catalase 9.2 per cent at 26°C.
Arterial pressure 126 mm. Hg. Exceedingly sensitive, especially to sounds. Placed under full ether.
Venous pressure: Ext. jug. 8.5 cm., "effective" 5.0 cm. H_2O.
5:18 Arterial pressure 126 mm. Hg, pulse 150, respiration 36.
5:45 Arterial pressure 110 mm. Hg, pulse 156, respiration 32.
Venous pressure: Ext. jug. 6.25 cm., "effective" 6.0 cm. H_2O.
Alkaline reserve 49.0 per cent.
6:25 Arterial pressure 126 mm. Hg.
6:45 Arterial pressure 126 mm. Hg, pulse 144, respiration 28.
Venous pressure: Ext. jug. 6.0 cm., "effective" 5.25 cm. H_2O.
Alkaline reserve 50.5 per cent.
Catalase 7.5 cc. at 25.5°C.
6:55 Sacrificed by intravenous injection of ether.
Injected with formalin. Brain and upper cord preserved in formalin.
Lungs showed solid-red areas similar to those previously noted.
Left tympanic membrane ruptured with blood in external auditory canal. Right membrane intact.

Dog 9 not used.

Dog 10. March 4, 1918. Male, medium sized.

7:50 a.m. One grain morphia hypodermically.
9:08 Operation completed under light ether.
9:25 Arterial pressure 100 mm. Hg, pulse 51, respiration 24.
Venous pressure: Ext. jug. 3.25 cm. "effective" 14.5 cm., Fem. 6.25 cm. H_2O.
Catalase 12 cc. at 25.5°C., 772 mm. Hg.
Alkaline reserve 43.5 per cent.
10:09 Exposed 10 feet in front of 12-inch rifle, firing excess charge.
10:13 Alkaline reserve 47.2 per cent.
Catalase 11 cc. at 23.5°.
Arterial pressure 70 mm. Hg, pulse 93, respiration 9.
Venous pressure: Ext. jug. 10.0 cm., "effective" 5.0 cm., Fem. 15.0 cm. H_2O.
Bleeding from mouth.
10:39 Dead.
Post mortem: Lungs very extensively hemorrhagic. This condition together with respiration and venous pressure indicate death by pulmonary hemorrhage. Formalin injected into carotid. Brain and upper cord preserved in formalin.

Dog 11. March 17, 1918. Female, large.

7:00 a.m. One grain morphia hypodermically.
8:12 Operation completed without ether.
8:20 Arterial pressure 130 mm. Hg, pulse 52, respiration 42.
Venous pressure: Ext. jug. 1.0 cm., "effective" 12.5 cm., Fem. 4.75 cm. H_2O.
Alkaline reserve 39.7 per cent.
Catalase 13.5 cc. at 24.75°C.
9:18 Exposed 100 feet in front of 12-inch rifle.
9:32 Catalase 14.5 cc. at 24°C.
Arterial pressure 125 mm. Hg, pulse 68, respiration 54.
Venous pressure: Ext. jug. 0.0 cm., "effective" 6.0 cm., Fem. 8.25 cm. H_2O.
10:11 a.m. Exposed 50 feet in front of 12-inch rifle.
10:20 Alkaline reserve 33.1 per cent.
Catalase 14.5 cc. at 23.5°C.
Arterial pressure 90 mm. Hg, pulse 256, respiration 34.
Occasional sighing respiration.
Venous pressure: Ext. jug. −1.5 cm., "effective" 13.0 cm., Fem. 5.0 cm. H_2O.
10:55 Arterial pressure 94 mm. Hg, pulse 216, respiration 34.
Venous pressure: Ext. jug. 1.5 cm., "effective" 5.0 cm., Fem. 4.5 cm. H_2O.
11:25 Arterial pressure 100 mm. Hg, pulse 240, respiration 36.
Venous pressure: Ext. jug. 2.5 cm., "effective" 6.0 cm., Fem. 6.0 cm. H_2O.
11:50 Arterial pressure 106 mm. Hg, pulse 224, respiration 40.
11:55 Sacrificed.
Post mortem. Lungs showed hemorrhagic areas. No specimens preserved.

Dog 14. March 11, 1918. Female, medium size.

12:00 m. One grain morphia hypodermically.
1:30 p.m. Arterial pressure 130 mm. Hg, pulse 76, respiration 15.
Femoral venous pressure 3.5 cm. H_2O.
Catalase 14.5 cc. at 23°C.
2:29 Exposed 20 feet in front of 12-inch rifle.
2:36 Catalase 15.4 cc. at 23.5°C.
Arterial pressure 15 mm. Hg, pulse 12, respiration 8.
Femoral venous pressure 15 cm. H_2O.
2:45 Dead. Slight hemorrhage from nose on return to laboratory.
No post mortem. No specimens saved.

Dog 15. March 11, 1918. Male, weight 34 pounds.

Morphia-ether anesthesia.
Exposed 10 feet in front of 12-inch rifle.
Dying on return to laboratory. Bloody-froth from mouth.
No post mortem. No specimens preserved.

Dog 16. March 12, 1918. Male, weight 31 pounds.

7:20 a.m. One grain morphia hypodermically.
7:50 One-half grain morphia hypodermically.
9:05 Arterial pressure 130 mm. Hg.
Femoral venous pressure 5.25 cm. H_2O.
9:55 Exposed 17 feet in front of 12-inch mortar.
10:05 No change in arterial pressure.
10:41 Exposed as at 9:55.
11:00 No change in arterial pressure.
11:30 Arterial pressure 120 mm. Hg.
1:00 p.m. Arterial pressure 138 mm. Hg.
Femoral venous pressure 5.25 cm. H_2O.
2:00 Exposed 4 feet from 2 pounds T.N.T.
2:15 No change in arterial pressure.
Femoral venous pressure 5.8 cm. H_2O.
Bloody froth in trachea.
3:18 Exposed 3 feet from 2 pounds T.N.T.
3:30 Arterial pressure 120 mm. Hg.
Femoral venous pressure 4.0 cm. H_2O.
4:20 Arterial pressure 100 mm. Hg.
Femoral venous pressure 4.75 cm. H_2O.
4:25 Sacrificed.
Post mortem. Lungs hemorrhagic. Formalin injected. Brain and upper cord preserved in formalin.
Both tympanic membranes ruptured.

Dog 20. March 15, 1918. Female, weight 29 pounds.

12:45 p.m. One grain morphia hypodermically.
1:45 One-half grain morphia hypodermically.
2:30 Arterial pressure 142 mm. Hg.
Femoral venous pressure 2.5 cm. H_2O.
Catalase 46.0 cc. at 23°C.

4:59 p.m. Exposed 15 feet in front of 10-inch rifle and 5 feet to windward side (wind 60 miles per hour).
5:08 Arterial pressure 134 mm. Hg.
Femoral venous pressure 5.0 cm. H_2O.

March 16, 1918.

7:00 a.m. Standing up. Wags tail. Looks well and quite bright.
One-half grain morphia hypodermically.
8:00 Arterial pressure 125 mm. Hg, pulse 184, respiration 20.
Femoral venous pressure 3.0 cm. H_2O.
Catalase 60 cc. at 23°C.
9:15 Exposed as at 4:59, March 15.
Arterial pressure 95 mm. Hg.
9:40 Exposed 13 feet in front of 10-inch rifle.
9:55 Arterial pressure 86 mm. Hg.
Femoral venous pressure 2.0 cm. H_2O.
10:10 Arterial pressure 75 mm. Hg, pulse 228, respiration 48.
10:30 Apparently dying. Chloroform.
Post mortem. Lungs widely hepatized, especially at roots and diaphragm. Bleeding from nose and mouth.
No specimens preserved

Dog 21. March 16, 1918. Female, weight 32 pounds.

10:00 a.m One and a half grains morphia hypodermically.
11:00 Arterial pressure 145 mm. Hg.
Catalase 57.0 cc. at 24.5°C.
11:26 Exposed 15 feet in front of and 3 feet to side of 10-inch rifle.
11:35 Arterial pressure 90 mm. Hg.
Catalase 58.0 cc. at 25°C.
Bleeding at mouth.
Sacrificed.
No post mortem. No specimens preserved.

Dog 22. March 5, 1919. Male, weight about 12 pounds.

9:00 a.m. One grain morphia hypodermically.
9:45 Catalase, 60.8 cc.
9:55 Venous pressure, femoral, 5.7 cm. H_2O.
10:00 Arterial pressure, 90 mm. Hg.
10:28 Catalase, 70.8 cc.
11:00 Exposed 12 feet in front of 10-inch rifle. Elevation 10°. Powder charge 156 pounds 8 ounces. Pressure 3 feet before dog, 97 pounds; 3 feet beyond dog, 126 pounds; on either side of dog, 140 pounds and 129 pounds per square inch. Average pressure of concussion about 130 pounds per square inch.
11:11 Catalase 102 cc.
11:23 Venous pressure, femoral, 5.5 cm. H_2O.
11:26 Arterial pressure 90 mm. Hg. No change in heart rate.
11:35 Catalase 91.2 cc.
1:10 p.m. Sacrificed. No post mortem.

Dog 23. Not used.

Dog 24. March 13, 1919. Female, weight 10 pounds.

7:50 a.m.	One-half grain morphia hypodermically.
8:40	One-half grain morphia hypodermically.
9:30	Arterial pressure 90 mm. Hg.
9:40	Venous pressure, femoral, 9.3 cm. H_2O.
9:45	Catalase 32.5 cc.
1:10 p.m.	One-half grain morphia hypodermically.
3:55	Exposed 11 feet in front of 12-inch mortar. Powder charge 37 pounds. Concussion pressure 388 pounds per square inch.
4:10	Arterial pressure 105 mm. Hg. No change in heart rate.
4:15	Venous pressure, femoral, 10.5 cm. H_2O.
7:00	Placed on ether bottle.
7:05	Arterial pressure 110 mm. Hg.
7:10	Venous pressure, femoral, 13 cm. H_2O.
7:15	Sacrificed—ether intravenously.
7:20	Post mortem. Bloody froth in trachea. Heart valves intact. Lung, heart and liver tissue in 10 per cent formalin. Hemorrhagic areas in lungs very extensive and certainly as prominent as seen in shocked animals.

Dog 25. Not used.

Dog 26. March 18, 1919. Male, weight 11 pounds.

7:45 a.m.	One-half grain morphia hypodermically.
9:00	Blood volume determined as 449 cc.
9:05	Catalase 45.5 cc.
9:08	Arterial pressure 85 mm. Hg. Venous pressure, femoral, 9.5 cm. H_2O.
9:39	Exposed 18 feet in front of 10-inch rifle. Powder charge, three-fourths normal. Concussion pressure 67.58 pounds per square inch.
9:45	Arterial pressure 90 mm. Hg.
10:40	Exposed 18 feet in front of 10-inch rifle. Powder charge, uniformity. Concussion pressure 319.5 pounds per square inch.
11:10	Arterial pressure 80 mm. Hg.
11:17	Blood volume determined as 458 cc.
11:40	Arterial pressure 35 mm. Hg.
11:43	Venous pressure, femoral, 7.3 cm. H_2O.
11:45	Catalase 24.9 cc.
11:55	Dead. No post mortem.

Dog 27. March 18, 1919. Female, weight 15 pounds.

12:30 p.m.	One grain morphia hypodermically.
1:15	Arterial pressure 120 mm. Hg.
1:20	Venous pressure, femoral, 10 cm. H_2O.
1:43	Exposed 15 feet in front of 10-inch rifle. Concussion pressure 281 pounds per square inch.
1:54	Arterial pressure 98 mm. Hg. Heart rate increased.
1:57	Venous pressure 8.8 cm. H_2O.
2:06	Bloody froth in nostrils.
2:10	Venous pressure 9.2 cm. H_2O.

2:12 p.m. Arterial pressure 103 mm. Hg.
2:27 Venous pressure 8.8 cm. H_2O.
2:29 Arterial pressure 107 mm. Hg.
2:42 Venous pressure 9 cm. H_2O.
2:43 Arterial pressure 100 mm. Hg.
2:45 Sacrificed—ether intravenously.
2:50 Post mortem. Lungs extensively hemorrhagic. Heart valves intact. Lung and heart tissue in 10 per cent formalin.

Dog 28. March 19, 1919. Male, about 12 pounds.

7:45 a.m. One grain morphia hypodermically.
8:30 Catalase 47 cc.
8:40 Alkaline reserve 56.4 per cent.
8:43 Arterial pressure 100 mm. Hg.
8:45 Venous pressure, femoral, 10.5 cm. H_2O.
9:53 Exposed 20 feet in front of 12-inch rifle. Concussion pressure 267 pounds per square inch.
9:56 Back to laboratory. Blood in nose.
10:00 Catalase 45.5 cc.
10:05 Alkaline reserve 43.8 per cent.
10:10 Arterial pressure 40 mm. Hg.
10:12 Venous pressure 9.8 cm. H_2O.
10:22 Breathing rapidly. No blood at nose or mouth.
10:39 Arterial pressure 60 mm. Hg.
10:40 Venous pressure 9.5 cm. H_2O.
10:50 Catalase 48.9 cc.
10:55 Arterial pressure 50 mm. Hg.
10:56 Venous pressure 9.6 cm. H_2O.
10:58 Alkaline reserve 38.9 per cent.
11:13 Venous pressure 11.3 cm. H_2O.
11:16 Arterial pressure 45 mm. Hg.
11:27 Dying gasps. Blood from nose.
11:30 Dead.
11:35 Post mortem. Lungs show usual hepatization. Heart valves intact. Abdomen clear. Tissue from lung, heart, brain, liver, stomach, intestine, spleen, kidney, stellate and coeliac ganglia and adrenal saved in Kaiserlin solution.

Dog 29. June 27, 1918. Female, small.

8:00 a.m. One grain morphia hypodermically.
10:30 One grain morphia hypodermically.
1:45 p.m. Arterial pressure 110 mm. Hg, pulse 80, respiration 16.
Femoral venous pressure 2.5 cm. H_2O.
Catalase.
2:45 Exposed 18 feet in front of 8-inch rifle.
Arterial pressure 105 mm. Hg, pulse 128, respiration 16.
Femoral venous pressure 7.0 cm. H_2O.
Catalase increased 0.3 cc.
11:00 (June 28, 1918.)
Arterial pressure 120 mm. Hg, pulse 150, respiration 14.
Sacrificed.
Post mortem. Lungs perfectly clear.

BIBLIOGRAPHY

(1) Mott: Lancet, 1916, i, 331.
(2) Mairet and Durante: Revue Neurol., 1919, xxvi, 97.
Carver and Dinsley: Brain, 1919, xlii, 113.
(3) Burge and Neill: This Journal, 1918, xlv, 246.
(4) Van Slyke: Journ. Biol. Chem., 1917, xxx, 347.
(5) Mairet and Durante: Presse Medicale, August 16, 1917.
(6) Crile: Official report to the Surgeon General.
(7) Mott: Lancet, 1916, i, 441.
(8) Commander: Revue Gen. d. Sci., April 15, 1916, 213.
(9) Dale and Laidlaw: Journ. Physiol., 1919, lii, 355.
(10) Wiggers: This Journal, 1918, xlv, 485.
(11) Penfield: This Journal, 1919, xlviii, 121.
(12) Porter and Turner: This Journal, 1916, xxxix, 236.
(13) Githens and Meltzer: This Journal, 1919, xlix, 120.
(14) Erlanger, Gesell and Gasser: This Journal, 1919, xlix, 99.
(15) Cannon: Journ. Amer. Med. Assoc., 1918, lxx, 611.
(16) Wallace, Frazer and Drummond: Lancet, 1917, ii, 727.
(17) Porter and Quinby: This Journal, 1908, xx, 500.
(18) Seelig and Lyon: Journ. Amer. Med. Assoc., 1908, lii, 45.
(19) Morison and Hooker: This Journal, 1915, xxxvii, 86.
(20) Erlanger, Gesell and Gasser: This Journal, 1919, xlix, 90.
(21) Meek and Gasser: This Journal, 1918, xlvii, 302.
(22) Hooker: This Journal, 1918, xlvi, 591.
(23) Hill: Caisson sickness, London, 1912.
(24) Porter and Emerson: Boston Med. and Surg. Journ., 1918, clxxix, 273.
(25) McKibben: This Journal, 1919, xlviii, 331.
(26) Gatti: Riforma Medica, 1918, xxxiv, 102.
(27) Cramer: Arch. d. Mal. du Coeur, etc., 1919, xi, 66.
(28) Mott: Lancet, February 12, 1916.
(29) Mott: Brit. Med. Journ., May 19, 1917.
(30) Mott: Journ. Roy. Army Med. Corps, 1917, xxix, 662.

THE GLUCOSE EQUIVALENT OF INSULIN IN DEPANCREATED DOGS

FRANK N. ALLAN

From the Department of Physiology, The University of Toronto[1]

Received for publication September 17, 1923

The pharmacological assay of insulin is proving a difficult problem to solve. The original unit was defined as that amount which lowers the blood sugar to the convulsive level of 0.045 per cent within four hours in normal rabbits weighing about 2 kgm. (1). Although this has so far proved itself to be the most reliable basis of assay it is not entirely satisfactory, so that other methods are being investigated. One of these is based on a determination of the number of grams of glucose which a given quantity of insulin can cause to be metabolized in the diabetic animal. This is called the glucose equivalent of insulin and it is measured by comparing the daily glucose balance of one period during which a certain amount of insulin is given with that of another period during which the amount of insulin is changed. A method based on this principle has been used in the clinic but with results that are not entirely satisfactory, dependent in part, no doubt, on the fact that the diabetic patient still retains some remnant of islet tissue capable of producing insulin. Since the amount of this endogenous insulin is unknown and is probably somewhat variable from day to day, the effect brought about by the insulin administered cannot be properly estimated. It is inaccurate to base the assay on the amount of insulin required to make the diabetic animal sugar-free, because it is impossible, in the absence of any sugar in the urine, to know whether an excess of insulin may not have been given.

To avoid this complicating factor we have tested the value of this method on completely depancreated dogs in whom there can be no question of an endogenous production of insulin and the present paper records some of the results which have so far been obtained. Although the investigation is not yet completed and we are not prepared to formulate any practical rule for the assay of insulin based on the above principle, several facts of fundamental importance have revealed themselves and we have judged it advisable to place these on record, especially since they have an undoubted application in the therapeutic use of insulin.

[1] The expenses of this investigation have been defrayed by the research grant of the University of Toronto and the Carnegie Corporation.

The first step in determining whether this method is to be a reliable one for measurement of the dosage of different batches of insulin is to see whether different amounts of the same batch given to a depancreated animal kept on constant diet of meat and sugar will cause proportionate variations in the carbohydrate balance. It is with this question that the present paper is concerned.

Method: Two female dogs weighing about 16 and 18 pounds respectively were used, and after pancreatectomy and Falck's operation they were placed in metabolism cages. The operations were performed with the usual aseptic technique, the animals recovering quickly and the wounds healing readily under insulin treatment.

They were fed with a diet consisting of a mixture of fresh chopped meat freed from visible fat, and cane sugar. This diet was found by preliminary trial to be the most satisfactory since in the absence of the pancreatic digestive juices, fat and, to a less extent, polysaccharide carbohydrate were found to be poorly digested. Although the liberal ration of meat was not entirely assimilated as revealed by the nitrogen balance, calculated from the nitrogen in the urine, yet the quantity given enabled the animals to maintain, or to gain, in body weight. The feces had a normal appearance and were not markedly offensive. The food was always taken with a relish and the animals were in good spirits and in good health during the period of observation which has now extended over four months.

The food was given in equal quantities in the morning and in the afternoon and the insulin was injected subcutaneously immediately before each feeding, except where noted otherwise.

The urine and cage washings were collected in a bottle, containing toluol, placed under the cage and catheterization was performed at the same hour each morning in order to obtain the complete twenty-four hour excretion.

The sugar in the urine was determined by Benedict's and Shaffer and Hartman's methods and the total nitrogen excretion by the Kjeldahl method. Qualitative tests for acetone bodies were also made.

The insulin used was manufactured either by the Connaught Laboratories of the University of Toronto or by the Eli Lilly Co. and a large enough quantity was obtained at a time to last for several weeks so that errors dependent upon variation in potency of different preparations might be avoided. Three lots of insulin were used in the experiments reported here, and each was said to contain 20 clinical units per cubic centimeter, according to the rabbit assay, a unit in this case being one-fifth of the original unit as defined in the first paragraph of this paper. It was kept in the refrigerator.

Results. These are given in tabular form. In each table the glucose metabolized is calculated as the difference between the total glucose intake and the excretion of glucose in the urine. Since cane sugar was given, its

weight was multiplied by 1.053 to give the amount of glucose ingested and to this was added the glucose derived from protein, which was obtained by multiplying the excretion of nitrogen in the urine by 3.625. No allowance is made for the glucose derived from fat, the fat content of the diet being kept as low as possible because of its defective digestion, due to the pancreatectomy. Since only 10 per cent of glucose could come from this source it is plain that no serious error is involved through this omission.

In table 1 (A and B) are given the results on a dog, I, that was depancreated on April 4 and was fed a daily ration of 600 grams meat with 150

TABLE 1A

Dog I. Insulin 789

Diet 600 grams meat and 150 grams sucrose

DATE	WEIGHT IN POUNDS	TOTAL N	GLUCOSE FROM PROTEIN	GLUCOSE FROM CH IN FOOD	TOTAL GLUCOSE INTAKE	GLUCOSE EXCRETED	GLUCOSE METABOLIZED	INSULIN		GLUCOSE EQUIVALENT PER UNIT
								Cubic centimeters	Units (clinical)	
April										
16	15–12		20.0	157.9	177.9	31.7	146.2	0.8	16	9.1
17	15–14	5.63	20.0	157.9	177.9	19.6	158.3	1.2	24	6.6
18	16–1	4.57	16.0	157.9	173.9	9.3	164.6	1.6	32	5.1
19		4.07	15.0	157.9	172.9	3.6	169.3	1.6	32*	5.3
20	16–9	5.29	19.0	157.9	176.9	9.0	167.9	1.6	32	5.2
21	16–9	4.11	15.0	157.9	177.9	2.2	174.7	1.6	32‡	5.5
22	16–10		20.0	157.9	177.9	2.2	175.7	1.6	32	5.5
23	16–12	6.13	22.0	157.9	179.9	16.5	163.4	1.2	24§	6.8

* 1.0 cc. in a.m. 0.6 cc. in p.m.

‡ 3 doses. Not complete urine.

§ 0.8 cc. in a.m. 0.4 cc. in p.m.

grams sucrose from April 16 to 23, after which the quantity was reduced to 100 grams. Similar observations were made simultaneously with the same insulin on another somewhat smaller dog, J, receiving 500 grams of meat and 100 grams sucrose daily and the results are collected in table 2. Taking the results of tables 1B and 2 together, and omitting for the present those of 1A, it will be seen that usually somewhat less than one-half of the nitrogen ingested as protein reappeared in the urine. This is due no doubt to faulty intestinal digestion and absorption although the feces were not visibly abnormal in character. No attempt was made in these particular experiments to bring about a closer protein balance, the above quantities of meat being chosen because it was found by trial that the animals thrived well on them and, as a rule, gained somewhat in weight. Of course we must recognize that with so much protein remaining unab-

sorbed in the intestine it is possible also that all of the cane sugar of the diet was not absorbed. Although this would introduce an error into

TABLE 1B

Dog I. Insulin 789

Diet 600 grams meat and 100 grams sucrose

Date	WEIGHT IN POUNDS	TOTAL N	GLUCOSE FROM PROTEIN	GLUCOSE FROM CH IN FOOD	TOTAL GLUCOSE INTAKE	GLUCOSE EXCRETED	GLUCOSE METABOLIZED	INSULIN		GLUCOSE EQUIVALENT PER UNIT
								Cubic centimeters	Units (clinical)	
April										
24		7.67	28.0	105.3	133.3	23.4	109.3	1.0	20	5.4
25		6.39	23.0	105.3	128.3	66.9*	61.4	0.6	12	5.1
26	16–8	7.30	26.0	105.3	131.3	33.3	98.0	1.2	24	4.1
27	16–12	6.81	24.0	105.3	129.3	5.3	124.0	1.4	28	4.4
28	16–12	6.84	25.0	105.3	130.3	5.4	124.9	1.6	32	3.9
29	16–12		(25.0)	105.3	130.3	26.6	103.7	1.2	24†	4.3
30	16–13	7.54	27.0	105.3	132.3	9.3	123.0	1.2	24	5.1
May										
1	16–11	7.51	27.0	105.3	132.3	10.0	122.3	1.2	24	5.1
2	16–11	7.46	27.0	105.3	132.3	14.6	112.4	1.0	20‡	5.9
3	17–2	9.51	34.0	105.3	139.3	91.5	47.8	0.4	8§	5.7
4	16–10	6.63	24.0	105.3	129.3	5.6	123.7	1.4	28	4.4
5	16–11	8.10	29.0	105.3	134.3	8.2	126.1	1.3	26¶	4.9
6	16–15		27.0	105.3	132.3	9.1	123.2	1.2	24	5.1
7	16–15	7.80	27.0	105.3	132.3	4.5	127.8	1.3	26	4.9
8	16–15	8.27	29.0	105.3	134.3	23.1	111.2	1.2	24	4.6
9	16–15	7.98	29.0	105.3	134.3	32.3	102.0	1.2	24△	4.3
10	16–15	6.37	22.0	105.3	127.3	21.8	105.5	1.0	20	5.3
11	17–1		24.0	105.3	129.3	27.4	101.9	0.8	16	6.4
12	16–12	7.46	27.0	105.3	132.3	47.6	84.7	0.6	12	7.1
13	16–10		27.0	105.3	132.3	75.0	57.3	0.4	8	7.2
14	16–12	7.80	28.0	105.3	133.3	12.1	121.2	1.4	28	4.3
15	16–12			105.3		(35.4)□		1.3	26	
16	16–0	8.09	29.0	105.3	134.3	16.3	118.0	1.2	24	4.9
17	16–15	7.79	28.0	105.3	133.3	21.4	111.9	1.2	24	4.7
18	17–0	8.95	32.0	105.3	137.3	33.9	103.4	1.0	20	5.2
19	16–12	9.08	33.0	105.3	138.3	37.0	101.3	0.93	18.6	5.4

* Not complete urine. Dog vomited.
† 0.8 cc. in a.m. 0.4 cc. in p.m.
‡ 0.6 cc. in a.m. 0.4 cc. in p.m.
§ 1 dose in a.m. only.
¶ 0.7 cc. in a.m. 0.6 cc. in p.m.
△ 2nd. dose of insulin not given until 2 hours after feeding.
□ 22 hour sample, time of feeding changed.

the determination of the absolute glucose equivalents of different samples of insulin it cannot seriously affect the comparative values of different doses of the same insulin, which is the problem of the present investigation.

The following conclusions may be drawn from the results: *1.* The glucose equivalent of the same dose of insulin given on different days varies within a relatively narrow range. Thus, from table 1B it is seen that with a daily injection of 1 cc. insulin in equal doses twice daily at the time of feeding the unit values were: 5.4, 5.9, 5.3 and 5.2; average 5.54; with 1.2 cc. they were: 4.1, 4.3, 5.1, 5.1, 5.1, 4.6, 4.3, 4.9 and 4.7; average 4.7. From table 2 it is seen that 1.8 cc. gave values of 3.0, 3.1, 2.8 and 3.2; average 3.02.

TABLE 2

Dog J. Insulin 789

Diet 500 grams meat and 100 grams sucrose

DATE	WEIGHT	TOTAL N	GLUCOSE FROM PROTEIN	GLUCOSE FROM CH IN FOOD	TOTAL GLUCOSE INTAKE	GLUCOSE EXCRETED	GLUCOSE METABOLIZED	INSULIN		GLUCOSE EQUIVALENT PER UNIT
								Cubic centimeters	Units	
May										
7	14–0	4.94	18.0	105.3	123.3	14.2	109.1	1.8	36	3.0
8	14–12	4.64	16.0	105.3	121.3	22.3	99.0	1.6*	32	3.1
9	14–12	5.02	18.0	105.3	123.3	10.3	113.0	1.8	36	3.1
10	15–0	6.44	23.0	105.3	128.3	28.1	100.2	1.8	36	2.8
11	14–8	6.17	22.0	105.3	127.3	13.4	113.9	1.8	36	3.2
12	15–0	5.29	19.0	105.3	124.3	12.3	112.0	1.6	32	3.5
13	15–0		(20.0)	105.3	125.3	13.6	111.7	1.4	28	4.0
14	14–15		(20.0)	105.3	125.3	5.8	119.5	1.4	28	4.3
15	15–2		(20.0)	105.3	125.3	20.3	105.0	1.3	26	4.0
16	15–0	7.29	26.0	105.3	131.3	5.1	126.2	1.2	24	5.3
17		8.12	29.0†	105.3	134.3	35.0	99.3	1.2	24	4.1
18	15–0	9.19	33.0	105.3	128.3	30.7	97.6	1.0	20	4.9
19	15–0	7.31	26.0	105.3	131.3	18.2	113.1	1.0	20	5.7

* Second injection of insulin not given until 2 hours after afternoon feeding.
† Dog vomited part of feeding.

2. The glucose equivalents for small doses of insulin are larger than for large doses. This is shown clearly in the curves of figure 1 from which it appears that within the range of the doses given the glucose equivalent is very nearly inversely proportional to the dose of insulin.

From the results of table 1A compared with those of 1B it is also seen, *3*, that the equivalent is decidedly greater when the amount of ingested carbohydrate is increased. Thus, with 1.2 cc. of insulin the glucose equivalent rose from an average of 4.7, when 100 grams sucrose were ingested, to 6.7 with 150 grams; and when 1.6 cc. insulin was used it rose from 3.9 (1 determination) with 100 grams sucrose, to 5.3 (average of 5 determinations) with 150 grams. These differences are also shown in the chart of figure 1, the small circles showing the values for 150 grams sugar and the dots and crosses those for 100 grams.

It might be questioned whether the dog was really assimilating the entire amount of sucrose given in the observations of table 1A. If the sugar were not absorbed from the intestine, or were excreted in the urine as sucrose, the amount of sugar considered to be metabolized, and therefore the sugar value per unit, would be erroneously high. The former possibility seems unlikely on account of the extreme readiness with which sugar is absorbed from the intestine. The latter possibility was ruled out as no change was found to occur in the reducing power of the urine after boiling with acid.

The different values observed for the same dose of insulin may be partly due to inequality in the rate of absorption of insulin and in this connection

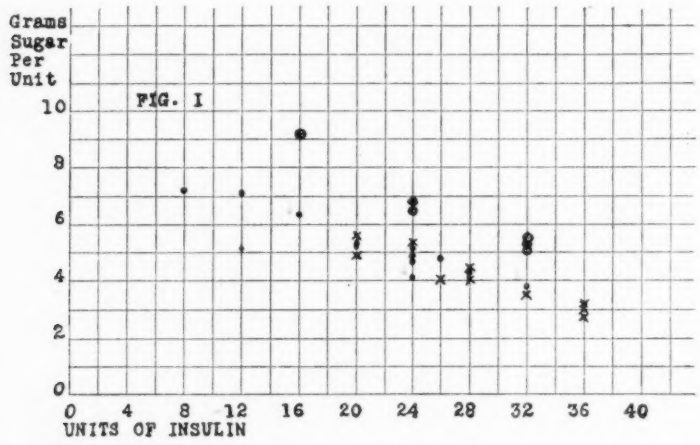


Fig. 1. Insulin 789. Glucose equivalents for dog I on 100 grams sugar represented by dot; on 150 grams represented by circle; for dog J on 100 grams represented by cross.

it is interesting to note that the glucose equivalent tends to be higher when the daily amount of insulin is given equally with the forenoon and afternoon feedings than when most is given in the forenoon.

To avoid these possible sources of error the observations have been repeated on the same animals with two other batches of insulin and greater care has been taken to see that the injections were always made in equal quantities immediately preceding the forenoon and afternoon feeding periods. In tables 3 and 4 are given the results with insulin 211. The two animals were observed simultaneously and from May 20 to May 28 they were given the same daily doses of insulin. After this date the daily dosage was not always the same for both animals. When insulin was omitted in

one of the dogs, I (June 7) it is seen that the glucose excretion was practically the same as the total glucose intake. It was probably greater, indicating a negative sugar balance, since some of the urine was lost. It is of interest that even with extremely small injections of insulin the utilization of carbohydrate became considerable, e.g., with 0.2 cc. insulin 86 out of 149 grams of glucose were metabolized on one occasion and later

TABLE 3

Dog I. Insulin 211

Diet 600 grams meat and 100 grams sucrose

DATE	WEIGHT	TOTAL N	GLUCOSE FROM PROTEIN	GLUCOSE FROM CH IN FOOD	TOTAL GLUCOSE INTAKE	GLUCOSE EXCRETED	GLUCOSE METABOLIZED	INSULIN		GLUCOSE EQUIVALENT PER UNIT
								Cubic centimeters	Units	
May										
20	16–13	7.41	26.8	105.3	132.1	5.5	126.6	1.6	32	4.0
21	16–15	8.84	32.1	105.3	137.4	9.4	128.0	1.6	32	4.0
22	17–1	6.87	24.8	105.3	130.1	1.7	128.4	2.0	40	3.2
23	17–3	7.95	28.6	105.3	133.9	3.8	130.1	1.8	36	3.6
24	17–0		(28.0)	105.3	133.3	1.7*	131.6	1.6	32	4.1
25	17–0		(28.0)	105.3	133.3	17.3	116.0	0.8†	16	7.2
26	17–8	7.89	28.6	105.3	133.9	3.3	130.6	1.6	32	4.1
27			(28.0)	105.3	133.3	3.0	130.3	1.4	28	4.7
28	18–0	7.32	26.4	105.3	131.7	2.0	129.7	1.4	28	4.6
29	18–0	9.81	34.4	105.3	140.1	2.6	137.5	1.2	24	5.7
30	17–12	8.37	30.3	105.3	135.6	23.8	111.8	0.4	8	14.0
31	17–8	9.02	32.5	105.3	137.8	22.2	115.6	0.8	16	7.2
June										
1	17–6	12.00	43.6	105.3	148.9	62.4	86.5	0.2	4	21.6
2	17–8	8.00	28.8	105.3	134.1	14.3	119.8	1.0	20	6.0
3	17–8		(28.0)	105.3	133.3	12.9	120.4	0.8	16	7.5
4	17–8	7.77	28.0	105.3	133.3	12.9	120.4	0.8	16	7.5
5	17–10	9.19	33.2	105.3	137.5	56.8	80.7	0.2	4	20.2
6	17–8	9.02	32.8	105.3	138.1	18.5	119.6	1.0	20	6.0
7	15.0	10.30	37.4	105.3	142.7	139.2‡	3.5	0	0	0

* Cage washings lost.

† Only one injection of insulin given this day.

‡ A small quantity of urine lost. Acetone bodies appeared in the urine.

80 out of 137 grams. The high glucose equivalents of these small doses of insulin is a point of considerable therapeutic importance and it would seem to lend support to the practice, in the treatment of diabetes in man, of combining control of the diet with the administration (perhaps by inunction) of small doses of insulin.

The conclusions drawn from the observations of tables 1 and 2 are seen to be confirmed by the results shown in tables 3 and 4. The latter lend

themselves better for a study of the relationship between the glucose equivalent and the amount of injected insulin, since there is a greater variation in dosage. This relationship is shown in the curve of figure 2 in which the results of table 3 are given in dots and those of table 4 in crosses. When logarithms of the coördinates are plotted the points fall a straight line

TABLE 4

Dog J. Insulin 211

Diet 500 grams meat and 100 grams sucrose

DATE	WEIGHT	TOTAL N	GLUCOSE FROM PROTEIN	GLUCOSE FROM CH IN FOOD	TOTAL GLUCOSE INTAKE	GLUCOSE EXCRETED	GLUCOSE METABOLIZED	INSULIN		GLUCOSE EQUIVALENT PER UNIT
								Cubic centimeters	Units	
May										
20	15-0	7.31	26.4	105.3	131.7	6.1	125.6	1.6	32	3.9
21	15-0	8.27	30.0	105.3	135.3	7.3	128.0	1.6	32	4.0
22	15-1	7.66	27.8	105.3	133.1	3.5	129.6	2.0	40	3.2
23	15-2	8.74	31.8	105.3	137.1	4.2	132.9	1.8	36	3.7
24	15-4		(28.0)	105.3	133.3	4.2*	129.1	1.6	32	4.0
25	14-8		(28.0)	105.3	133.3	43.9	89.4	0.8¶	16	5.6
26	15-1	7.74	28.0	105.3	133.3	10.5	122.8	1.6	32	3.8
27			(28.0)	105.3	133.3	‡		1.4	28	
28	15-4	7.71	27.8	105.3	133.1	6.6	126.5	1.4	28	4.5
29	15-8	8.19	29.8	105.3	135.1	3.7	131.4	1.4	28	4.7
30	15-8	7.38	26.6	105.3	131.9	3.5	128.4	1.8	36	3.6
31	15-8	8.39	30.4	105.3	135.7	1.7†	143.0	1.2	24	5.6
June										
1		9.13	33.0	105.3	138.3	22.3	116.0	0.6	12	9.7
2	15-5	7.28	26.4	105.5	131.7	6.4	125.3	1.0	20	6.3
3	15-8		(28.0)	105.3	133.3	9.1	124.2	1.0	20	6.2
4	15-8	9.46	34.4	105.3	139.7	20.4	119.3	0.8	16	7.5
5	15-8	7.96	28.6	105.3	133.9	26.3	107.6	0.6	12	9.0
6	15-8	8.88	32.2§	105.3	137.5	9.3	122.9	1.0	20	6.4
7	15-4	6.49	23.6	105.3	128.9	13.4†	110.2	0.8	16	7.2

* Cage washings lost.

† Not complete 24 hr. quantity.

‡ Urine lost.

§ Dog vomited part of feeding.

¶ Only one injection of insulin given this day.

which is represented by the equation log. $g = 1.86 - 0.85$ log. u; which may be expressed in the form $g(u)^{0.85} = 10^{1.86}$ where u, is the number of units of insulin given and g, the number of grams of sugar metabolized for each unit. By using this equation the glucose equivalents have been calculated for each dose of insulin and compared in table 5 with those actually found. They correspond very closely except for the smallest doses of insulin (4 units) when the obtained values are considerably below the cal-

culated, perhaps because an insufficient amount of the hormone was present in the body to prevent breakdown of the glycogen which had become stored in large amounts in the liver as a result of the previous treatment with insulin and sugar.

TABLE 5

Glucose equivalents of insulin 211 as calculated from equation $g(u)^{0.55} = 10^{1.86}$

	DATE	DOSE	GLUCOSE EQUIVALENT	
			Obtained	Calculated
Dog I	May 20	32	4.0	4.1
	May 21	32	4.0	4.1
	May 22	40	3.2	3.4
	May 23	36	3.6	3.7
	May 24	32	4.1	4.1
	May 25	16	7.2	7.4
	May 26	32	4.1	4.1
	May 27	28	4.7	4.6
	May 28	28	4.6	4.6
	May 29	24	5.7	5.2
	May 30	8	14.0	13.3
	May 31	16	7.2	7.4
	June 1	4	21.6	23.9
	June 2	20	6.0	6.1
	June 3	16	7.5	7.4
	June 4	16	7.5	7.4
	June 5	4	20.2	23.9
	June 6	20	6.0	6.1
	June 7	0		
Dog J	May 20	32	3.9	4.1
	May 21	32	4.0	4.1
	May 22	40	3.2	3.4
	May 23	36	3.7	3.7
	May 26	32	3.8	4.1
	May 28	28	4.5	4.6
	May 29	28	4.7	4.6
	May 30	36	3.6	3.7
	June 1	12	9.7	9.4
	June 2	20	6.3	6.1
	June 3	20	6.2	6.1
	June 4	16	7.5	7.4
	June 5	12	9.0	9.4
	June 6	20	6.4	6.1

The curve calculated from the equation is drawn along with the observed values in figure 2. The assay of insulin may be made by a modification of this curve in which the grams of glucose metabolized are plotted against units of insulin. Further work is in progress to test the practical value of this method.

In tables 6 and 7 are given the results obtained with insulin 917. From June 8 to June 21 the dogs were given fairly large doses of insulin. Dog I made a good gain in weight, and dog J was also in good condition. From

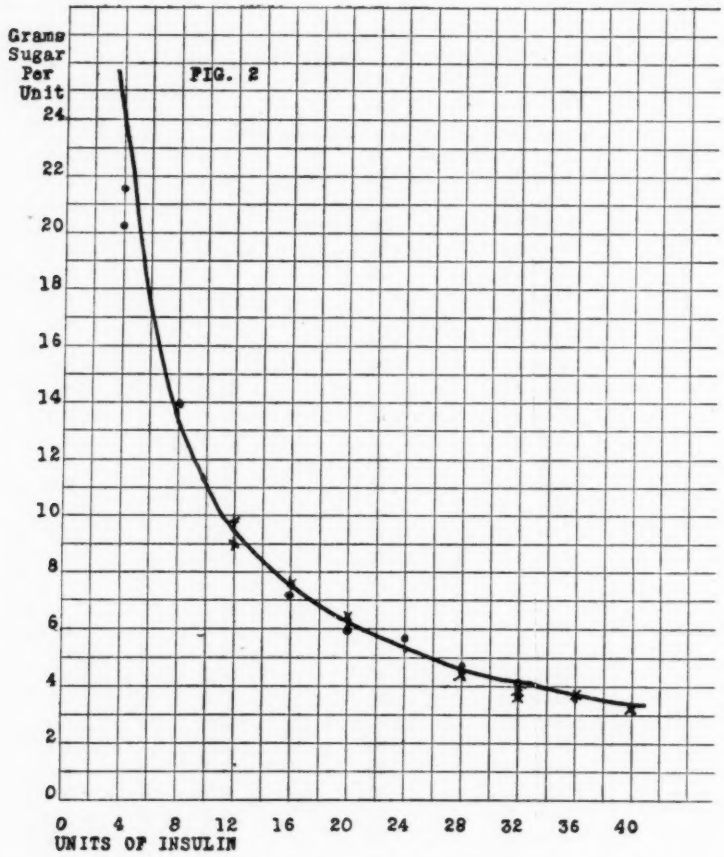


Fig. 2. Insulin 211. Glucose equivalents for dog I represented by dot; for dog J represented by cross.

June 22 to 29 the daily amounts of insulin were kept at 20 units, while the amount of sugar in the diet was varied from 50 to 150 grams per day. From June 30 to July 9 the dose was kept at 32 units while the amount of sugar given was varied as before.

TABLE 6

Dog I. Insulin 917

DATE	WEIGHT	TOTAL N	GLUCOSE FROM PROTEIN	GLUCOSE FROM CH IN FOOD	TOTAL GLUCOSE INTAKE	GLUCOSE EXCRETED	GLUCOSE METABOLIZED	INSULIN		CH EQUIVALENT
								Cubic centimeters	Units	
June										
8	16–12	7.45	27.0	105.3	132.3	35.0	97.3	1.2	24	4.2
9	16–9		(27.0)	105.3	130.0	35.3	97.0	1.2	24	4.0
10	16–12		(27.0)	105.3	130.0	19.4	110.6	1.6	32	3.5*
11	17–1	5.77	20.7	105.3	126.0	13.4	112.6	1.6	32	3.5†
12	17–3	7.52	27.2	105.3	132.5	60.4	72.1	1.4	28	2.6‡
13	17–1	8.14	29.6	105.3	134.6	27.8	116.8	1.6	32	3.6
14	17–1	8.68	31.6	105.3	136.9	30.8	106.1	1.6	32	3.3
15	17–8	9.32	33.8	105.3	139.1	32.2	106.9	1.8	36	3.0
16	17–6	9.87	35.8	105.3	141.1	30.4	110.7	1.8	36	3.1
17	17–11		(34.0)	105.3	139.3	18.9	110.4	1.8	36	3.1
18	17–11	8.26	29.8	105.3	135.1	16.9	118.2	2.0	40	3.0
19	17–15	8.40	30.4	105.3	135.7	5.5	130.2	2.0	40	3.3
20	17–14	7.95	28.6	105.3	133.9	11.6	122.3	2.0	40	3.1
21	17–15	7.96	28.6	105.3	133.9	13.9	120.0	2.0	40	3.0
22	17–15	8.15	29.6	105.3	134.9	17.9	117.0	1.0	20	5.8
23	17–12	8.04	29.2	105.3	134.5	19.2	115.3	1.0	20	5.8
24	17–12		(30.0)	52.6	82.6	12.7	69.9	1.0	20	3.5
25	17–11	8.71	31.6	52.6	84.2	5.0	79.2	1.0	20	3.9
26	18–0	8.86	32.1	157.9	190.0	22.0	168.0	1.0	20	8.4
27	18–2	7.74	27.9	157.9	185.8	23.5	162.3	1.0	20	8.1
28	17–8	9.15	33.0	157.9	190.9	54.0	136.9	1.0	20	6.8
29	18–0	10.06	36.4	157.9	194.3	57.0	137.3	1.0	20	6.9
30	17–14		(33.0)	157.9	190.3	46.0	144.3	1.6	32	4.5
July										
1	17–15		(33.0)	157.9	190.3	33.3	157.0	1.6	32	4.9
2	17–14	7.65	27.8	105.3	133.1	15.0	118.1	1.6	32	3.7
3	17–13	7.88	28.5	105.3	130.6	3.2	130.6	1.6	32	4.1
4	17–9	7.99	28.8	52.6	81.4	3.2	78.2	1.6	32	2.4
5	17–4	8.08	29.2	52.6	81.8	0	81.8	1.6	32	2.6§
6	17–0	5.38	19.6	131.6		5.0		1.6	32	
7	17–1	7.80	28.1	131.6	159.7	4.1	155.6	1.6	32	4.9
8	17–4		(28.0)	131.6	159.6	4.8	154.8	1.6	32	4.8
9	17–8		(28.0)	105.3	133.3	2.2	131.1	1.9	38	3.4

* 24½ hour period.

† 23½ hour period.

‡ First injection of insulin not given until 5 hours after the morning feeding.

§ Dog vomited part of feeding.

TABLE 7

Dog J. Insulin 917

DATE	WEIGHT	TOTAL N	GLUCOSE FROM PROTEIN	GLUCOSE FROM CH IN FOOD	TOTAL GLUCOSE INTAKE	GLUCOSE EXCRETED	GLUCOSE METABOLIZED	INSULIN		CH EQUIVALENT
								Cubic centimeters	Units	
June										
8	15-8	7.85	28.4	105.3	133.7	19.8	113.9	1.2	24	4.7
9	15-9		(28.0)	105.3	133.3	14.4	118.9	1.2	24	5.0
10	15-8		(26.0)	105.3	131.3	15.5	115.8	1.6	32	3.6
11	15-5	7.19	26.0	105.3	131.3	9.2	122.1	1.6	32	3.8
12	15-8	8.48	30.8	105.3	136.1	72.7	63.4	1.4	28	2.3
13	15-8	9.05	32.8	105.3	138.1	9.8	128.3	1.6	32	4.0
14	15-8	9.08	32.9	105.3	138.2	11.0	127.2	1.6	30	4.0
15	15-9	9.55	34.5	105.3	139.8	17.5	122.3	1.8	36	3.4
16	15-9	9.24	33.5	105.3	138.8	17.4	121.4	1.8	36	3.4
17	15-12		(34.0)	105.3	139.3	17.5	121.8	1.8	36	3.3
18	15-15	9.01	32.7	105.3	138.0	5.6	132.4	2.0	40	3.3
19	15-11	8.48	30.8	105.3	136.1	15.2	120.9	2.0	40	3.0
20	15-7	8.96	32.4	105.3		(29.9)		2.0	40	
21	15-5	7.77	28.0	105.3		8.8		2.0	40	
22	15-8	9.24	33.5	105.3	138.8	18.2	120.6	2.0	40	3.0
23	15-5	9.84	35.8	105.3	141.1	38.3	102.8	1.0	20	5.1
24	15-7		(35.0)	105.3	140.3	34.2	106.1	1.0	20	5.3
25	15-8	11.08	40.2	52.6	92.8	33.9	58.9	1.0	20	2.9
26	15-1	10.11	36.5	52.6		31.1		1.0	20	
27	14-11	9.32	33.8	52.6	85.4	35.9	49.5	1.0	20	2.5
28	14-4	9.25	33.5	157.9		65.3		1.0	20	
29	15-0	9.49	34.4	157.9	192.3	47.5	144.8	1.0	20	4.2
30	15-3	9.22	33.5	157.9	191.4	42.1	149.2	1.0	32	4.7
July										
1	15-5		(33.0)	157.9	190.9	27.4	163.5	1.0	32	5.1
2	15-8	8.86	32.0	105.3	137.3	25.8	111.5	1.6	32	3.5
3	14-12	8.61	31.2	105.3	136.5	26.5	110.0	1.6	32	3.4
4	14-9	8.68	31.6	52.6	84.2	14.6	69.6	1.6	32	2.5
5	14-12	9.56	34.5	52.6	87.1	7.1	80.0	1.6	32	2.5
6	14-11	10.68	38.8	131.6	170.4	25.0	145.4	1.6	32	4.5
7	14-9	7.22	26.0	131.6		10.1		1.6	32	
8	14-8		(33.0)	131.6	164.6	8.6	156.0	1.6	32	4.9
9	14-12		(33.0)	105.3	138.3	2.5	131.8	2.1	42	3.1

June 8. Fed 500 grams meat. 100 grams sucrose.

June 10. 24½ hour period.

June 11. 23½ hour period.

June 12. First injection of insulin not given until 5 hours after the morning feeding. Ration increased to 600 grams meat with 100 grams sucrose.

June 14. Small amount of injection lost.

June 20. Small amount of injection lost.

June 21, 26, 28, July 7. Small quantity of feeding vomited.

June 29, 30, July 1. Small quantity of urine lost.

The results may be summarized as follows:

Dog I. Fed 600 grams meat and 100 grams sucrose.

INSULIN GIVEN PER DAY (CLINICAL UNITS)	GLUCOSE EQUIVALENT OBTAINED
20	5.8, 5.8
24	4.1, 4.0
32	(3.5), (3.5), 3.6, 3.3, 3.7, 4.1.
36	3.0, 3.1, 3.1
38	3.4
40	3.0, 3.3, 3.1, 3.0

Insulin 20 clinical units per day

SUGAR GIVEN PER DAY	GLUCOSE EQUIVALENT OBTAINED
50 grams	3.5, 3.9
100 grams	5.8, 5.8
150 grams	8.4, 8.1, 6.9, 6.9

Insulin 32 clinical units per day

SUGAR GIVEN	GLUCOSE EQUIVALENT
50 grams	2.4, 2.6
100 grams	3.7, 4.1
125 grams	4.9, 4.8
150 grams	4.5, 4.9

Dog J. Diet containing 100 grams sucrose daily

INSULIN GIVEN PER DAY	GLUCOSE EQUIVALENT
20 units	5.1, 5.3
24 units	4.7, 5.0
32 units	(3.6),(3.8), 4.0
36 units	3.4, 3.4, 3.3
40 units	3.3, 3.0, 3.0

Insulin 20 units per day

SUGAR GIVEN	GLUCOSE EQUIVALENT
50 grams	2.9, 2.5
100 grams	5.1, 5.3
150 grams	(7.2) (7.5), 8.2

Insulin 32 units per day

50 grams	2.2, 2.5
100 grams	3.5, 3.4, 3.1
125 grams	4.5, 4.9
150 grams	(4.7), (5.1)

(Figures with parentheses are not quite accurate for reasons mentioned in footnotes of tables.)

The same general conclusions regarding the relationship of dosage and the amount of ingested sugar to glucose equivalents can be drawn as in the previous observations, and it will be observed, as would be expected, that with large doses of insulin (between 36 and 40 units) the glucose equivalent of each unit comes to be practically constant at least within the limits of experimental error.

The influence of the amount of ingested sugar on the equivalent is also clearly shown in these results and it will be seen that with 32 units in both animals there is no further change beyond 125 grams of sugar.

TABLE 8

NUMBER INSULIN	UNITS INJECTED	GLUCOSE EQUIVALENTS	
		Dog I	Dog J
789	20	5.45 (av.)	5.7 (1 determination) 4.1 (1 determination)
	24	4.7 (av.)	5.3
	28	4.4, 4.4, 4.3	4.0, 4.3
	32	3.9	3.1, 3.5
	36	3.02 (av.)	
211	20	6.0, 6.0	6.3, 6.2,6.4
	24	5.7	5.6
	28	4.7, 4.6	4.5, 4.7
	32	4.0, 4.0, 4.1, 4.1	3.9, 4.0, 4.0, 3.8
	36	3.6	3.7, 3.6
917	20	5.8, 5.8	5.1, 5.3
	24	4.2, 4.0	4.7, 5.0
	28		
	32	3.6, 3.3	3.6, 3.8
	36	3.0, 3.1, 3.1	3.4, 3.3, 3.4

Comparison of the glucose equivalent for the three batches of insulin used in these observations shows them to be tolerably uniform when the ingestion of sucrose is the same, as is illustrated in table 8.

Discussion. The fact that each unit of insulin can bring about the metabolism of a greater amount of carbohydrate when the dose given is small than when it is large indicates that in the latter case there is an excess of insulin in relationship to the available number of glucose molecules for it to act upon. This may be because a large part of the insulin is excreted in the urine but it is possible also that it is due to the fact that the action of insulin on glucose metabolism proceeds according to the same laws as govern enzyme action, namely, that the amount of substrat acted on is not a linear function of the amount of enzyme except when there is a large relative excess of substrat. This would mean, in connection with the effect of insulin, that when the amount of glucose available in the body

is large compared with the dose of insulin the amount of glucose actually metabolized is nearly proportional to the dose and is large for each unit. On the other hand, when the insulin is relatively in excess, less and less extra glucose can be metabolized for each increase in unitage, since the number of molecules of glucose available for each unit to act upon becomes progressively smaller. The practical application of these facts in the treatment of diabetes with insulin has already been alluded to.

By increasing the amount of available glucose, as by increasing the ingestion of sucrose in these experiments, the same dose of insulin causes more to be metabolized per unit. There is a limit to which this can occur, however, which is probably due to the fact that insulin acts as a co-enzyme and the limit to its effect will be set by the amount of enyzme available.

Apart from these theoretical considerations the present results have a practical bearing in that they show no value can be attached to the glucose equivalent of insulin in the treatment of diabetes unless the level of the carbohydrate balance is taken into consideration. A small dose of insulin will cause a much larger amount of glucose to be metabolized per unit than a large dose. Failure to recognize this fact probably explains in part the considerable variations in glucose equivalents obtained by various clinical investigators. Taking the most dependable of these, published by Wilder, Boothby, et al (2) the glucose equivalent per unit was found to run between the extremes of 0.9 and 3.1 with an average of 1.45. Although much of this variation no doubt depends on the facts that different patients and different samples of insulin were used, it is altogether likely that much of it is due to a failure to consider the carbohydrate balance. According to Woodyat (2), the equivalent works out at 1.0 to 1.5 grams with considerable consistency. These values for the human diabetic are considerably less than the average of those obtained in the present investigation on diabetic dogs even when the lowest values are taken. This difference may be due in part to differences in body weight. It is possible also that lower values would be obtained in the observations in dogs if the carbohydrate were restricted to that derived from protein. Our reason for not having as yet done this is that we desire to maintain the animals in good condition and we have found that this is best secured by allowing them some cane sugar in the diet.

CONCLUSION

1. The glucose equivalent of insulin, that is, the number of grams of glucose which each unit can cause to be metabolized, determined on two depancreated dogs fed with meat and cane sugar, is found to vary with the dose given and with the amount of carbohydrate in the diet.

2. When the carbohydrate ingestion is constant the glucose equivalents per unit become progressively smaller as the dose is increased and an equation is given to show the relationship.

3. When the amount of insulin injected is constant and the carbohydrate is increased the glucose equivalent per unit of insulin is increased up to a certain limit beyond which it remains constant.

4. The practical application of these facts in the clinical use of insulin is indicated.

In conclusion I wish to express my appreciation for the advice and assistance given by Prof. J. J. R. Macleod.

BIBLIOGRAPHY

(1) Banting, Best, Collip, Macleod and Noble: This Journal, 1922, lxii, 162.
(2) Wilder, Boothby, Barborka, Kitchen and Adams: Journ. Metabolic Research 1922, ii, 701.
Woodyat: Ibid., 793.

STUDIES ON FACTORS PRODUCING A RAPID INCREASE OR DECREASE IN THE NUMBER OF RED AND WHITE CELLS IN THE BLOOD STREAM

I. Acids and Bases

ERNEST F. BOSTROM

From the Department of Physiology, University and Bellevue Hospital Medical College, New York

Received for publication September 17, 1923

In a previous paper (1) the writer has presented evidence to show that a redistribution of cells is at least partly responsible for the red cell increase observed in certain experimental conditions, the number of erythrocytes augmenting in the skin vessels and at the same time decreasing in the blood of the heart. The cause of the accumulation in the cutaneous area was sought in changes on the surface of the red cells and of the endothelial cells lining the smaller vessels making these cells more adhesive (or less repellent) and thereby increasing the tendency of the red corpuscles to collect in the narrower paths of the circulation.

The present task is to examine the extent and cause of the redistribution of the blood cells, including the leucocytes, in conditions where rapid numerical cell changes have been observed, and also to determine other factors that may cause a total or relative increase or decrease of the blood cells. Recent advances in colloidal chemistry have suggested that the problem may be approached from a new angle. The behavior of the blood cells must be dependent largely on surface phenomena, explainable on the basis of Donnan's law of equilibrium, the validity of which has been established by Loeb (2). The importance of the medium in determining the surface phenomena of the cells and colloids of the blood suggested the first set of experiments, namely, that of acid and alkali injections.

Literature. Very few data throwing any light on the effects of acids and alkalis on the numerical changes of the blood cells are to be found in the literature. Ciaccio (3) in studying the mechanism of the so-called digestion leucocytosis, gave dogs by mouth 50 to 100 cc. 0.4 per cent HCl. A rapid increase in the white cells followed. This increase Ciaccio explains as due to the formation of secretin or a similar substance which gets into the blood stream and eventually reaches the bone marrow where it acts as a stimulus. He believes that the HCl of the gastric juice is essen-

tial for the production of the digestion leucocytosis. This belief he bases on the above described experiments and also on the fact that pilocarpin, which is supposed to stimulate the secretion of gastric HCl, likewise produces a leucocytosis, while in cases of ahydrochloria, digestion leucocytosis is absent.

Ciaccio's idea of a hormone stimulating the bone marrow is not new. Downs and Eddy (4) found that subcutaneous injections of secretin, prepared in the usual way except that it was made acid by adding up to 2 volumes per cent glacial acetic acid, was followed by an increase of red as well as of white cells. Repeated injections caused a permanent rise in the leucocyte count while the increase in red cells was sooner or later followed by a decrease. The increase according to their opinion is due to a stimulation of the bone marrow.

Hirsch (5) has produced experimental data to show that the leucocytoses which accompany experimental infections are preceded by a lowered alkali reserve. He suggests that in some way the bone marrow is stimulated to greater activity under those conditions.

Description of experiments and observations. The method for cell counting was the usual one, described in a preceding paper (1). Behrens' (6) modification of Hayem's diluting fluid for red cell count was used. The modification consists in adding a little acetic acid to the Hayem formula, thus preventing the red cells from settling in the counting pipettes. As diluting fluid for white cells a 1 per cent solution of acetic acid was used except after $NaHCO_2$ injections when a 5 per cent solution of the acid was found necessary in order to prevent the cells from agglutinating. Whenever possible, a 2 per cent solution of novocaine was substituted for a general anesthetic. Medium sized dogs and cats were employed. The animals had been without food about twenty hours at the time of the experiments. Seventeen experiments dealing with the effects of acids and bases on the blood count have been performed. In order to save space only 4 charts are shown, the results presented not deviating essentially from those of the other experiments.

The results of the acid injections all agree in one respect, namely, in showing a decrease in red as well as in white cells in the heart blood. This can be explained either by assuming that cells have been removed from the blood stream or destroyed, or that they are held back in some narrower paths of the circulation. The evidence is largely in favor of the last possibility. When the acid is injected subcutaneously, an increase of red as well as of white cells is soon noticed in the skin vessels. After intravenous injections, however, there is a decrease of white cells in the same area. The cause of this can be inferred from the results presented in chart 4 (*0–45*), showing that the number of white cells may at times become very high in the liver and in the spleen as compared with other

parts of the body. It may be mentioned in this connection that Wells (7) has found the leucopenia which follows bacterial injections to be due to an accumulation of leucocytes in the internal organs.

The results presented in chart 4 (at *0*) indicate that the white cells either normally are unequally distributed in the blood stream, or else easily accumulate in certain parts of the circulation in response to changing conditions. It is possible that in this case the ether or the laparotomy preceding the blood count is responsible for the unequal distribution of the white cells at the start of the experiment. In agreement with the findings of Wells (7), the white count from the right ventricle is higher than that from the left. Wells explains his results as due to the irritating effect of the ether, which causes a retention of white cells in the lung vessels, decreasing the number that go to the left side of the heart. It is very likely that the ether is at least partly responsible for the retention in the lungs. The mechanism by which this is brought about will be further discussed below and in a following paper.

Evidently the red cells do not accumulate in the narrower paths of the circulation as readily as the leucocytes (see chart 3 at *0*). The difference may be due to the fact that the white cells normally keep closer to the walls of the vessels, as a result of their lower specific gravity. In the capillaries, however, even the red cells come in close touch with the vessel walls, and there at least the position of the cells must be of minor importance. It seems probable that the leucocytes naturally are more adhesive than the red cells and therefore more readily stick to the walls of the vessels.

The cutaneous red curve after intravenous injection of acid is characterized by sudden numerical changes (see chart 1). The cells come, so to speak, in bunches, the result of which is that the count may at one time be above normal and shortly afterward considerably below. Chart 3 shows (at *45* compared with at *0*) how the erythrocytes distribute themselves unequally after subcutaneous injection of acid. There is a great rise in the cutaneous count and a corresponding drop in the left and right heart. In this case as in the case of the white count (chart 4) the blood from the left heart contains fewer cells than that from the right. It is probable that the red cells also may under certain condition be held back in the parenchyma of the lungs. There is a slight decrease in the number of cells in the spleen. Judging from these results the accumulation of red cells after subcutaneous injection of acids takes place mainly in the cutaneous vessels.

Chart 3, in addition, indicates how the curves which represent red cell counts from various parts of the body after diverging as a result of the acid injection, again are made to converge (at *90*) by the injection of $NaHCO_3$. In other words the alkali causes an equalized distribution of the red cells. The same effect on the white cells is observed (chart 4

at *90*). It is of especial interest that the white cell curves converge not to the extent that the effect of the acid is wiped out, but also the effect of the ether, or whatever factor caused the unequal distribution at the start, is counteracted. This lends support to the idea that the mechanism of unequal distribution may be the same in most cases, involving the forma-

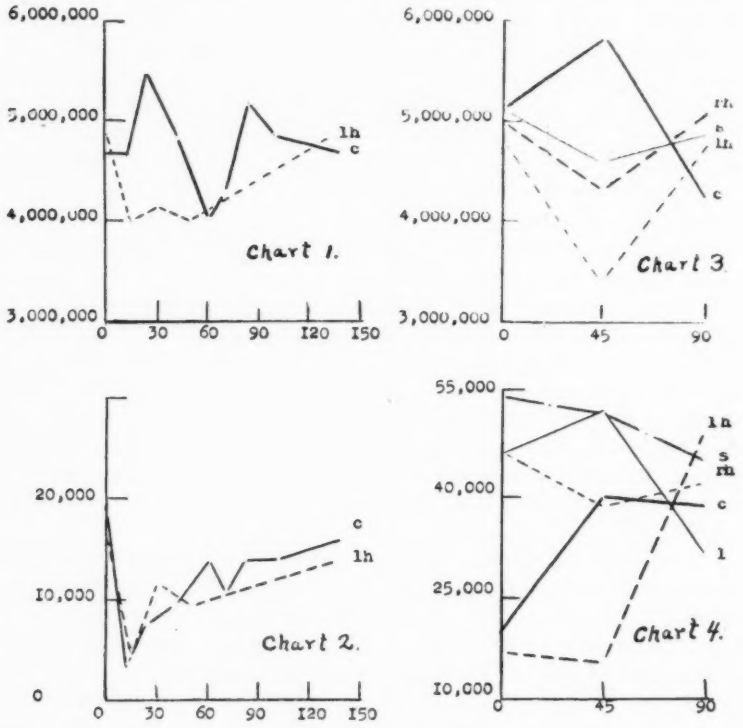


Chart 1. Erythrocyte count after intravenous injection of 10 cc. N HCl. Dog. No anesthetic. The figures along the ordinate indicate number of cells per cubic millimeter of blood, the figures along the abscissa are minutes after injection. *c* = cutaneous, *lh* = left heart.

Chart 2. Leucocyte count after intravenous injection of 10 cc. N HCl. Dog. No anesthetic. Figures as in chart 1.

Chart 3. Erythrocyte count after subcutaneous injection of 10 cc. HCl (*0-45*), and subcutaneous injection of 20 cc. 5 per cent $NaHCO_3$ (*45-90*). Cat. Ether anesthetic. The figures are arranged as in chart 1. The alkali was injected immediately after the count at *45*. *rh* = right heart, *s* = spleen, *l* = liver.

Chart 4. Leucocyte count after subcutaneous injection of 10 cc. of N HCl (*0-45*), and later of 20 cc. 5 per cent $NaHCO_3$ (given immediately after *45*.) Cat. Ether anesthetic. Figures arranged as in chart 1.

tion of acids which act on colloids (either present in the circulation, including the vessel walls, or else capable of readily diffusing into it) in such a way as to change them from the $R\begin{matrix}NH_2]\\COOH\end{matrix}$ type to the $R\begin{matrix}NH_2\\COOH]\end{matrix}$ type. This will be further discussed below.

That it is the adhesiveness of the cells rather than vasomotor changes that bring about the unequal distribution is made plain from the fact that counts from larger vessels, partly occluded by a tourniquet or by the introduction of a cannula, and from any cutaneous vessel, practically always run parallel. (They are not necessarily identical.) This was found to hold true in 10 cases out of 11, whether a rise or a drop in the cutaneous count was produced. Interesting in this connection are the observations of Duke in cases of pernicious anemia. He found that in those conditions the count from the finger is persistently higher than that from the cubital vein. However, if a tourniquet is kept on for some time previously to the withdrawing of the blood from the cubital vein, its cell content is found to increase rapidly, much more so than under ordinary conditions. It is easy to see how this can be explained by assuming an increased adhesiveness on the part of the red cells in this disease.

The possibility of the red blood cells varying greatly in adhesiveness has been demonstrated by *in vitro* experiments. A convenient way of measuring this property is to determine the rate at which the cells settle in a test tube. If care be taken to have the number of cells approximately the same, and the temperature constant, an increased rate of settling will show a greater adhesiveness on the part of the corpuscles, because under those conditions they tend to form aggregates which sink faster than the individual cells. This can be deduced from Stokes' equation,

$$v = \frac{2}{9} g (s_1 - s_2) \frac{I}{u} r^2$$

where v is the rate of sedimentaion, g the constant of acceleration, s_1 the specific gravity of the suspended particles, s_2 the specific gravity of the suspension fluid, r the radius of the suspended particles, u the coefficient of viscosity.

Fåhreus (9) has shown that only changes in the value of r are of any practical importance. In this case the radius of the individual cells probably remains quite constant. But if two cells adhere to each other, the effect on the rate of sedimentation will be approximately the same as if the radius had been doubled. It is, of course, apparent that the above formula cannot be used for mathematical determinations of the rate, because aggregates of corpuslces are probably very far from having a spherical form. But it will serve to illustrate what happens to the rate

when aggregates are formed, and it indicates that the adhesiveness of the red cells may be measured with a great deal of accuracy by the rate of settling.

Fåhreus (9) who has measured the rate of sedimentation under various conditions, found that the globulin fraction of the blood proteins increases the rate considerably. Höber (10) associates this with the position of the isoelectric point of globulin. Of most direct interest in this connection are the effects of acids. Fåhreus states that small quantities of acids have no effect on the rate of sedimentation. The following experiment corroborates this. Five sedimentation tubes containing 8 cc. of normal saline were treated with a 5 per cent solution of sodium bicarbonate, or with 0.1 N HCl so as to make the pH of the solutions in the tubes respectively 7.24, 7.0, 6.67, 6.61 and 6.57 before the addition of the corpuscles. To each of these tubes was added 0.5 cc. of washed red cells. The amount of sedimentation was ascertained after one hour by measuring the height of clear fluid in the upper part of the tubes. Since there was no difference in the amount of sedimentation, it follows that acids in small amounts are ineffective. The explanation for this is that, after the corpuscles had been in the suspension fluid for some time, the suspensions were all found to have the same pH. This result is, of course, due to the presence of buffer substances in the corpuscles. Evidently in this experiment we are not dealing with variations in the pH. This suggests that before acids can exert an effect on the rate of sedimentation of the red cells (or on the adhesiveness of the cells, which is practically the same thing), all buffer substances must be combined with acids.

In agreement with this, it was found that when larger quantities of acids were added, the effect on the adhesiveness of the corpuscles became marked. In the following experiment, different amounts of a 5 per cent $NaHCO_3$ solution and of 1/25 N HCl were added to 7 sedimentation tubes containing 8 cc. normal saline and 0.5 cc. washed corpuscles, each. The results are tabulated below.

TUBE NUMBER	AMOUNT OF ALKALI OR ACID ADDED	SUPERNATANT FLUID MEASURED IN MILLIMETERS AFTER				
		15 min.	30 min.	45 min.	120 min.	12 hrs.
1	0.3 cc. 5 per cent $NaHCO_3$	0	0	2	2	42
2	0.2 cc. 5 per cent $NaHCO_3$	0	1	2	2.5	42
3	0.1 cc. 5 per cent $NaHCO_3$	0	2	3	3.5	55
4		1	3	5	6	
5	0.1 cc. 1/25N HCl	55	58	58	58	58
6	0.15 cc. 1/25N HCl	25	34	38	42	42
7	0.25 cc. 1/25N HCl	2	3	3	3	3

It is apparent from these experiments that acids in sufficient amount to overcome the buffer action of the corpuscles will increase their adhesive power. A surplus of acids evidently inhibits sedimentation. The explanation of this in the case of tube 6 probably is that the corpuscles take on positive surface charges. In tube 7 some process similar to gel formation occurred which prevented sedimentation.

The question arises what bearing these results have on the *in vivo* experiments. It does not seem probable that 10 cc. NHCl injected into the jugular vein, as was done in some experiments referred to above, are sufficient to use up the buffers of the whole blood. But the acid must, even when injected directly into the blood stream, at first act more or less locally. It will naturally require some time for the mixing to take place. The buffers of a small part of the blood may thus be used up and the pH of the plasma around some cells and colloids change considerably toward the acid side, with the effect that these alter their electrical surface charges, i.e., colloids of the $R\begin{matrix}NH_2] \\ COOH\end{matrix}$ type (therefore negatively charged) will be converted into the $R\begin{matrix}NH_2 \\ COOH]\end{matrix}$ type (and take on a positive charge). It is easy to see how such a positively charged colloid can become the nucleus of an aggregation of corpuscles that have not been directly acted upon by the acid and therefore have their normal negative surface charges. For in this case, as in all cases where electrical charges are active, the law holds that "like repel and unlike attract."

To what extent a general decrease in the pH of the blood, small as it is under ordinary conditions, may affect the adhesiveness of the cells will at present have to be left undecided. There are, however, *in vitro* experiments which demonstrate that the transition from a positive to a negative (or vice versa) surface charge on colloids, is very abrupt, and if the pH of the suspension fluid is near the iso-electric point of the colloid, a very minute change in the pH of the fluid may cause the colloid to lose its normal surface charge and take on the opposite one. This may take place in the blood stream only if there are colloids present, the iso-electric point of which lies close to that of the blood (ordinarily about 7.4). If the blood became more acid, i.e., if its pH changed to about 7.2, the above-mentioned colloids would take on positive surface charges and act as nuclei round which the negatively charged corpuscles and colloids would collect. The presence in the blood of colloids having their iso-electric point at about 7.4 is possible although no definite data are available.

A more complete discussion of how colloids may affect the red and white blood count (by causing a redistribution of the cells and by "stimulating" the blood-forming organs) is reserved for a following paper.

That changes in the pH outside of the blood vessels may affect the white cell content of the blood, has been demonstrated by Gräff. He noticed that in some fetuses the zone outside the blood vessels of the umbilical cord, especially in the area between the blood vessels and the outer surface of the cord, was filled with leucocytes, while in others no white cells at all could be seen in the same area. It occurred to Gräff that variations in the pH of the embryonic fluids might account for the difference. Actual determinations lent support to this idea. He found that the more acid the embryonic fluid, the greater the migration of white cells out of the blood vessels. He also pointed to other facts demonstrating that leucocytes generally move from a higher to a lower pH. For instance, the pH of furuncles is ordinarily low, sometimes below 6.0. The leucocytes in the furuncles come, of course, from the blood,[1] having a pH of about 7.4. An additional proof that the leucocytes move from a higher to a lower pH is furnished by the fact that, as Gräff points out, in the decidua a collection of white cells often can be seen after normal delivery between the blood vessels and the side of the membrane that has been in contact with the uterine musculature. He explains this as due to acids formed in the musculature of the uterus during labor. In agreement with this explanation, no migratory leucocytes are found in the above-mentioned area if the fetus has been removed in some other way than through labor.

The experiments of Feringa (12) point in a similar direction. He was able to produce a migration of leucocytes into the abdominal cavity by injecting various substances intraperitoneally. That the migration of the white cells was associated with a lowering of the pH of the peritoneal exudate he demonstrated by determining the H ion concentration when migration occurred, invariably finding it increased under those conditions. On the other hand, if the acidification of peritoneal exudate was prevented through intraperitoneal injections of $NaHCO_3$, no leucocyte migration into the peritoneal cavity would take place.

Gräff's and Feringa's experiments lend support to the idea that acid formation is of the greatest importance in the production of leucocytosis. In agreement with this, the writer has found that procedures which ordinarily causes a rise in the white cell count—such as injection of blood into the peritoneal cavity (13), (14), intravenous injection of secretin (4) or pepton (unpublished)—are ineffective if a certain amount of $NaHCO_3$ is added. Similarly increases of the red cells can be prevented through $NaHCO_3$. The details of these experiments will be given in a following paper.

[1] In this discussion an increase in the H-ion concentration is sometimes expressed by saying that the pH is diminished, referring, of course, to the pH as generally written and not to its actual value.

SUMMARY

1. The injection of $NaHCO_3$ is always followed by an increase of red as well as of white cells in the heart blood. The increase in this part of the circulation is accounted for by a decrease in the cutaneous vessels and in the liver and spleen parenchyma.

2. The administration of acids has the opposite effect as far as the heart blood is concerned: the number of red and white cells is diminished. The cause of this is an accumulation of cells in the smaller vessels, either in the cutaneous area or in the parenchyma of internal organs, or in both. Very often it seems to be a question of where the cells are first caught, that part of the circulation showing an increase, the others a decrease.

3. The redistribution of the cells is attributed to a change in the adhesive power of the cells, alkalis causing this to become weaker, acids stronger.

The writer begs to acknowledge his indebtedness to Prof. H. C. Jackson whose criticism and advice during the progress of this work have been of the greatest value.

BIBLIOGRAPHY

(1) Bostrom: This Journal, 1921, lviii, 195.
(2) Loeb: Proteins and the theory of colloidal behaviour. New York, 1922.
(3) Ciaccio: Haematologica, iii, 1.
(4) Downs and Eddy: This Journal, 1918, xlvi, 209.
(5) Hirsch: Journ. Infec. Dis., 1921, xviii, 275.
(6) Behrens: Pflüger's Arch., 1922, cxcv, 18.
(7) Wells: Journ. Infec. Dis., 1917, xx, 219.
(8) Duke: Arch. Int. Med., 1922, xxx, 94.
(9) Fåhreus: Comm. de l'Inst. Serother. de l'Etat Danois, 1921, xi, 1.
(10) Höber: Klin. Wochenschr., 1922, i, 2412.
(11) Gräff: Münch. Med. Wochenschr., 1922, i, 1721.
(12) Feringa: Proc. Acad. Sci., Amsterdam, 1922, xxv, 36.
(13) Ghiron: Policlinico, Sezione Chirurgica, 1923, xxx, 137.
(14) Wright and Livingston: Unpublished.

ANTINEURITIC VALUE OF HOG MUSCLE

RALPH HOAGLAND

From the Biochemic Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.

Received for publication September 17, 1923

In view of the importance of vitamins in human nutrition, the need for adequate and correct information concerning the vitamin content of our common foodstuffs is apparent. The time-consuming methods at present required in the estimation of the vitamin content of a food product make the accumulation of reliable data on the subject very tedious. As a result there has been too great a tendency to assign positive vitamin values to foodstuffs on the basis of inadequate experimental evidence.

Meat, in particular, has suffered in this respect and until very recently, at any rate, it was considered as being very deficient in vitamins A, B and C. The opinions of several authorities on the subject follow:

McCarrison (1): In animal tissues this vitamin (B) is comparatively scanty, except in highly cellular organs such as the liver, kidney, brain, sweetbreads, fish-roe, and heart muscle. Actively-growing animal tissues do not contain it in appreciable amounts.

Sherman (2): Meat. From the distribution of vitamin B in the animal body, as indicated above, it is clear that ordinary meats are poor in this vitamin, while heart, liver and kidney contain it in somewhat larger proportion. Quantitative comparisons either of these meats with each other or with other foods, cannot readily be drawn from the data thus far available, but the experiments of Osborne and Mendel make it plain that ordinary meat is not to be depended upon to furnish the water-soluble vitamin needed for growth.

Funk (3): The significance of meat as a component of our diet is threefold. First, as an important source of protein; second, as a source of vitamins when it is used as a sole food; and third, for its "vitamin-sparing" action. The last characteristic will be treated more fully later. In accord with our experience, we know of no single food which requires so little vitamin addition as does protein material, especially meat.

McCollum (4): Osborne and Mendel have shown that muscle tissue is very deficient in the anti-neuritic factor, water-soluble B, and in the anti-ophthalmic factor, fat-soluble A, and that it contains but very little of the anti-scorbutic factor, water-soluble C.

In meat eating of the type so common in America, in which ham, steaks, roasts, chops, and other cuts which are derived from muscle, are almost exclusively used, the only important supplementing effect of meat on the remainder of the diet lies in the enhancement of the protein and in the increase in the phosphorus content.

Hoagland (5) appears to have been the first to report that voluntary-muscle tissue (of the hog) was relatively rich in the antineuritic vitamin. He states: "Meat may be regarded as an important source of vitamin B in the diet. Pork in particular—that is, the lean meat—is rich in this vitamin, comparing favorably in this respect with liver and kidney, organs heretofore recognized as containing an abundance of vitamin B. Beef appears to contain a much smaller portion of this vitamin, while mutton occupies an intermediate position."

Since the publication of the above report (5) the author has determined the antineuritic value of several additional samples of hog muscle and as the values obtained are much higher than those previously found, the results are herewith reported. For the sake of comparison the antineuritic values obtained for several lots of yeast are also submitted.

Experimental work. The methods employed were essentially those previously described by the author (5). The hog muscle was trimmed as free from fat and connective tissue as practicable, ground, mixed with water and toluol to form a thin paste, spread out in a thin layer on shallow pans and dried in a current of warm air at a temperature under 60°C. The dry muscle was ground, transferred to bottles and held in cold storage until needed.

The rice used as a base in all the rations was ordinary polished rice. In order to reduce its already low antineuritic value, the rice was ground and then heated 3 to 4 hours in an autoclave at 120°C.

Each ration was fed daily, except Sunday, to 4 healthy pigeons of the "Homer" type, the daily portion for each pigeon amounting to 5 per cent of the initial weight of the bird. The pigeons were weighed individually before feeding on the first day of the test and at weekly intervals thereafter. Forced feeding was practiced throughout the test, the ration being fed into the crop of the pigeon through a Gooch funnel, using a glass rod as a plunger.

The term "survival period" indicates the period between the start of the experiment and *a*, the development of positive symptoms of polyneuritis, or *b*, death of the bird, or *c*, the end of the test.

The care and feeding of the pigeons used in the tests reported in this paper were entrusted to Abraham Balter, formerly Laboratory Aid in the Biochemic Division, to whom thanks are due for his very efficient services.

Tests with check rations very deficient in antineuritic vitamin. In order to determine the survival period and change in weight of pigeons fed rations very deficient in the antineuritic vitamin, two pens of birds were fed autoclaved polished rice exclusively, while two other pens were fed autoclaved rice supplemented with autoclaved beef, ash mixture, and butter fat or cod liver oil, so as to make the rations adequate in every respect except for the antineuritic vitamin. The autoclaved beef was prepared by heating ground, dried, lean beef 3 hours in an autoclave at 120°C. to render it free from antineuritic vitamin. The results of this test are presented in table 1.

The results of the tests with pens 1 and 2, receiving only polished rice, are very similar to those previously obtained by the author (5). Pens 3 and 4, fed rations complete except for the antineuritic vitamin, gave results very much like those obtained with pens 1 and 2. The average

TABLE 1

Feeding tests with rations very deficient in antineuritic vitamin

RATION		PIGEON NUMBER	INITIAL WEIGHT	FINAL WEIGHT	CHANGE IN WEIGHT	SURVIVAL PERIOD	RESULT
Ingredients	Per cent		*grams*	*grams*	*per cent*	*days*	
Pen 1:							
Autoclaved rice	100	2061	366	348	−4.9	18	Polyneuritis
		2062	362	284	−21.5	18	Polyneuritis
		2064	316	255	−19.3	28	Polyneuritis
		2065	425	303	−28.7	25	Polyneuritis
Average			367	298	−18.6	22	
Pen 2:							
Autoclaved rice	100	249	425	383	−9.9	17	Polyneuritis
		250	470	273	−41.9	46	Polyneuritis
		251	389	282	−27.5	24	Polyneuritis
		252	419	306	−27.0	24	Polyneuritis
Average			426	311	−26.6	28	
Pen 3:							
Autoclaved rice	76	2066	305	280	−8.2	21	Polyneuritis
Autoclaved beef	15	2067	362	320	−11.6	21	Polyneuritis
Cod liver oil	5	2068	304	256	−15.8	27	Polyneuritis
Ash mixture	4	2069	300	225	−25.0	29	Polyneuritis
Average			318	270	−15.2	25	
Pen 4:							
Autoclaved rice	76	245	379	329	−13.2	21	Polyneuritis
Autoclaved beef	15	246	396	275	−30.6	42	Polyneuritis
Butterfat	5	247	377	304	−19.4	28	Polyneuritis
Ash mixture	4	248	335	311	−7.2	21	Polyneuritis
Average			372	305	−17.6	28	

survival period of pens 1 and 2 was 25 days, that of pens 3 and 4, 26½ days. The addition of autoclaved beef, cod liver oil or butter fat, and ash mixture to the rice diet fed pens 3 and 4 slightly reduced the loss in weight of the pigeons as compared with the birds fed rice exclusively.

Antineuritic value of yeast. Three lots of yeast were used in these tests. Yeast 815 was pure, compressed bakers' yeast obtained fresh from the

factory of one of the largest yeast manufacturers in this country. The product was dried overnight in a current of air at a temperature below 60°C.

Yeast 855 was pure bakers' yeast obtained in dry condition from another factory of the same manufacturer.

Yeast 862 was dry, powdered, autolyzed brewers' yeast obtained from a firm supplying vitamin preparations to the trade. The product furnished was reported to be the same as that which is used extensively abroad as a source of vitamin B for experimental and medicinal purposes. The results of the feeding tests with yeast are reported in table 2.

Five per cent of dried bakers' yeast 815 in the ration fed to pen 5 was insufficient to protect 4 pigeons against polyneuritis and loss in weight during the test period of 56 days, the average survival period being 50 days and the loss in weight 21.3 per cent. Ten per cent of the same lot of yeast in the ratio fed to pen 6 protected 4 pigeons against polyneuritis and only one bird lost slightly in weight.

Five per cent of dried bakers' yeast 855 in the ration fed to pen 7 failed to protect 3 pigeons against polyneuritis during the test period of 56 days, and although the fourth bird did not develop polyneuritis, it was in very poor condition at the close of the test, having lost 37.3 per cent in weight. The average survival period of this pen of pigeons is less, and the average loss in weight greater, than that of pen 5 receiving a ration containing 5 per cent of the other lot of bakers' yeast.

Four per cent of autolyzed brewers' yeast 862 in the otherwise adequate ration fed to pen 8 protected the pigeons against polyneuritis for an average period of only 36 days, and the average loss in weight was 10.8 per cent. Eight per cent of the same lot of yeast in a similar ration fed to 4 pigeons in pen 9 protected all the birds against polyneuritis, and 3 of them against loss in weight, the fourth losing 3.7 per cent. Three birds were in good condition and one was in fair condition at the close of the test. The average gain in weight of the birds was 8.8 per cent.

In general, the results of the tests with yeast indicate that 5 per cent of either lot of dried bakers' yeast, or 4 per cent of the dried brewers' yeast, in a ration was insufficient to protect a pen of 4 pigeons against polyneuritis and loss in weight during a period of 56 days. Ten per cent of dried bakers' yeast or 8 per cent of dried brewers' yeast in a ration was sufficient to protect a pen of 4 pigeons against polyneuritis and only two birds lost slightly in weight. It appears, then, that the minimum percentage of any one of the above lots of yeast that would have been necessary to protect a pen of pigeons against polyneuritis and loss in weight during a period of 56 days lies between 5 and 10 per cent.

Antineuritic value of hog muscle. The following described samples of hog muscle were tested for their antineuritic properties:

TABLE 2

Antineuritic value of yeast when fed to pigeons

RATION		PIGEON NUMBER	INITIAL WEIGHT	FINAL WEIGHT	CHANGE IN WEIGHT	SURVIVAL PERIOD	RESULT
Ingredients	Per cent		*grams*	*grams*	*per cent*	*days*	
Pen 5:							
Bakers' yeast 815	5	2071	326	232	−28.8	52	Polyneuritis
Autoclaved rice	95	2072	335	226	−32.6	52	Polyneuritis
		2073	351	295	−15.9	38	Polyneuritis
		2074	305	281	−7.9	56	Polyneuritis
Average			329	259	−21.3	50	
Pen 6:							
Bakers' yeast 815	10	2075	360	385	+6.9	56	Good condition
Autoclaved rice	90	2077	323	322	−0.3	56	Good condition
		2078	321	348	+8.4	56	Good condition
		2079	354	380	+7.3	56	Good condition
Average			340	359	+5.6	56	
Pen 7:							
Bakers' yeast 855	5	196	375	255	−32.0	24	Polyneuritis
Autoclaved rice	93	197	381	256	−32.8	43	Polyneuritis
Cod liver oil	2	199	313	216	−31.0	51	Polyneuritis
		1262	316	198	−37.3	56	Poor condition
Average			346	231	−33.3	44	
Pen 8:							
Brewers' yeast 862	4.0	295	340	339	−0.3	17	Polyneuritis
Autoclaved rice	69.5	296	362	337	−6.9	24	Polyneuritis
Autoclaved beef	12.5	297	400	350	−12.5	45	Polyneuritis
Cod liver oil	5.0	298	397	304	−23.4	56	Thin but active
Cottonseed oil	5.0						
Ash mixture	4.0						
Average			375	333	−10.8	36	
Pen 9:							
Brewers' yeast 862	8.0						
Autoclaved rice	67.7	299	318	400	+25.8	56	Good condition
Autoclaved beef	10.3	300	367	410	+11.7	56	Good condition
Cod liver oil	5.0	1247	352	339	−3.7	56	Fair condition
Cottonseed oil	5.0	1267	344	348	+1.2	56	Good condition
Ash mixture	4.0						
Average			345	374	+8.8	56	

Sample 816. Muscle from fresh pork hams purchased from a local packinghouse. No information as to how the hogs had been fed was available.

Samples 844 and 847 were obtained from the fresh hams from two hogs supplied by the Animal Husbandry Division of the Bureau of Animal Industry. The dam of the hogs was fed a mixture of 8 parts cornmeal, 4 parts wheat middlings, and 1 part fish meal while suckling her pigs. When 2 weeks old the young pigs were allowed access to shelled corn in a self-feeder, and 2 weeks later to wheat middlings also. This ration was fed until weaning time when the pigs were given free access to a self-feeder containing shelled corn, wheat middlings and fish meal in separate compartments. This ration was fed until time of slaughter.

TABLE 3

Composition of dried hog muscle

LABORATORY NUMBER	SOURCE OF MUSCLE	COMPOSITION		
		Moisture	Protein	Fat
		per cent	*per cent*	*per cent*
816	Ham	3.62	62.62	32.17
844	Ham	3.27	70.50	25.26
847	Ham	4.64	69.56	23.68
860	Loin	8.89	47.69	39.66

Sample 860 was the muscle from fresh pork loins supplied by the Animal Husbandry Division. The dam of the hogs from which the loins were derived was fed the same ration as the dam of the above lot of hogs. The young pigs were given access first to shelled corn until weaning time. The pigs were then fed shelled corn and fish meal in a self-feeder for a time and finally were hand-fed a mixture of 63 parts cornmeal, 31 parts peanut meal, and 6 parts cottonseed oil until time of slaughter.

The data in table 3 are presented for the purpose of making correction for the relatively high fat content of the samples of hog muscle so that the antineuritic value of the fat-free muscle tissue can be shown.

The data presented in table 4 indicate a much higher antineuritic value for hog muscle than that previously reported by the writer (5), but in that investigation the minimum proportion of hog muscle used in a ration was 15 per cent. The results of the tests are so clear-cut that but little discussion seems necessary. The percentage of dried hog muscle used in the several rations varies from 5 to 15, or 3.4 to 11.4 of the fat-free muscle. Seven rations were fed to 26 pigeons in 7 pens for a period of 56 days, and not a case of polyneuritis developed. Seventeen birds gained in weight, one to the extent of 20.4 per cent, one did not change, while 8 lost slightly,

TABLE 4

Antineuritic value of dried hog muscle fed to pigeons

RATION		PIGEON NUMBER	INITIAL WEIGHT	FINAL WEIGHT	CHANGE IN WEIGHT	SURVIVAL PERIOD	RESULT
Ingredients	Per cent		*grams*	*grams*	*per cent*	*days*	
Pen 10:							
Hog muscle 816	5.0						
Autoclaved rice	71.1						
Autoclaved beef	11.5	287	450	505	+12.2	56	Good condition
Cod liver oil	5.0	288	397	388	−2.3	56	Good condition
Cottonseed oil	3.4	1252	312	330	+5.8	56	Good condition
Ash mixture	4.0	1264	321	345	+7.5	56	Good condition
Average			370	392	+5.8	56	
Pen 11:							
Hog muscle 816	10.0	253	450	509	+13.1	56	Good condition
Autoclaved rice	90.0	254	440	475	+8.0	56	Good condition
		255	355	350	−1.4	56	Good condition
		256	440	427	−3.0	56	Good condition
Average			421	440	+4.2	56	
Pen 12:							
Hog muscle 844	10	257	426	419	−1.6	56	Good condition
Autoclaved rice	90	258	469	461	−1.7	56	Good condition
		259	472	479	+1.5	56	Good condition
		260	418	400	−4.3	56	Good condition
Average			446	440	−1.5	56	
Pen 13:							
		261	400	430	+7.5	56	Good condition
Hog muscle 844	15	262	354	374	+5.6	56	Good condition
Autoclaved rice	85	263	355	383	+7.9	56	Good condition
		264	380	440	+15.8	56	Good condition
Average			372	407	+9.2	56	
Pen 14:							
		265*	381	398	+4.5	22	Broken leg; removed
Hog muscle 847	10	266	324	390	+20.4	56	Good condition
Autoclaved rice	90	267†	329	338	+2.7	36	Died, not polyneuritis

* This pigeon suffered a broken leg on the 22nd day of the test and was chloroformed. The data for the bird are not included in the averages.

† This pigeon had a badly congested crop from the 31st to the 36th day of the test, when it died. Since the bird had gained 2.7 per cent in weight on the 28th day of the test, and there were no characteristic symptoms of polyneuritis, it was considered that death was probably due to an injury to the crop caused by forced feeding. The data for the pigeon are not considered in the averages.

TABLE 4—*Concluded*

RATION		PIGEON NUMBER	INITIAL WEIGHT	FINAL WEIGHT	CHANGE IN WEIGHT	SURVIVAL PERIOD	RESULT
Ingredients	Percent						
			grams	*grams*	*per cent*	*days*	
		268	285	310	+8.8	56	Good condition
Average			305	350	+14.6	56	
Pen 15:		269	317	345	+8.8	56	Good condition
Hog muscle 847	15	270	388	409	+5.4	56	Good condition
Autoclaved rice	85	271	386	392	+1.6	56	Good condition
		272	393	393	+0.0	56	Good condition
Average			371	385	+4.0	56	
Pen 16:							
Hog muscle 860	6.6	291	340	321	−5.6	56	Fair condition
Autoclaved rice	70.0	292	275	306	+11.3	56	Good condition
Autoclaved beef	12.0	293	357	378	+5.9	56	Good condition
Cod liver oil	5.0	294	325	302	−7.1	56	Fair condition
Cottonseed oil	2.4						
Ash mixture	4.0						
Average			324	327	+1.1	56	

the greatest loss being 7.1 per cent. The average results for the 7 pens show that 6 pens gained in weight from 1.1 to 14.6 per cent while one pen lost 1.5 per cent.

Particular attention is called to the results obtained from pens 10 and 16. The ration fed pen 10 contained only 5 per cent of dried hog muscle 816, or 3.4 per cent of fat-free muscle, and yet none of the birds developed polyneuritis and there was an average gain in weight of 5.8 per cent. The ration fed pen 16 contained only 6.6 per cent of dried hog muscle 860 or 4 per cent of the fat-free muscle, but none of the pigeons in this pen developed polyneuritis. Two birds gained 5.9 and 11.3 per cent, respectively, in weight while 2 lost 5.6 and 7.1 per cent, respectively, the pen showing an average gain in weight of 1.1 per cent.

SUMMARY

The antineuritic value of 2 samples of dried bakers' yeast, 1 sample of dried brewer's yeast, and 4 samples of hog muscle were determined by means of feeding tests with pigeons.

It was found that 5 per cent of either lot of bakers' yeast, or 4 per cent of the brewers' yeast, in a ration was not sufficient to protect pigeons against polyneuritis during a test period of 56 days, but 10 per cent of

bakers' yeast or 8 per cent of brewers' yeast was found to have full protective action.

The minimum percentage of each sample of hog muscle used in the several rations, viz., 5, 10, 10 and 6.6 per cent, respectively, each fully protected pigeons against polyneuritis during the test period.

BIBLIOGRAPHY

(1) McCarrison: Studies in deficiency diseases. 1921, 12.
(2) Sherman: The vitamins. 1922, 85.
(3) Funk: The vitamines. 1922, 262.
(4) McCollum: The newer knowledge of nutrition. 1922, 144–5.
(5) Hoagland: Bull. 1138, U. S. Department of Agriculture, 1923, 46.

VAGUS APNEA

WALTER J. MEEK

From the Physiological Laboratory of the University of Wisconsin Medical School

Received for publication September 17, 1923

With the increase in our knowledge of the rôle played by carbon dioxide in respiration, most recent writers have interpreted the respiratory stoppage which follows forced respirations as due entirely to a decreased blood content of CO_2 and have denied the existence of any such thing as vagus apnea. Such a conclusion has so far been justified in view of the fact that none of the experiments purporting to show a nervous apnea have eliminated gaseous changes in the blood. Haldane (1) may be consulted for this point of view as well as for the historical development of the subject.

The word "apnea" will be used in the original sense of Rosenthal, that is, a cessation of discharges from the respiratory center following a period of over-ventilation. The pauses produced during sustained inflation or deflation of the lungs are not included in this definition since, as Haldane has shown, they consist of prolonged inspiratory and expiratory effects due to the accumulating CO_2.

Since the work of Hering and Breuer it has been known that the afferent impulses coördinating the discharges of the respiratory center with the movements of the lungs are carried by the vagi. That these impulses consist in part at least of an inhibition of inspiratory muscles on distention of the lungs was shown by Head in his well-known experiments with the slip of the diaphragm.

The modern conception of respiratory regulation then assigns to CO_2 the rôle of a stimulus to the respiratory center in proportion to its content in the blood. It is this influence that determines the extent to which inflation or deflation must take place in order to bring into play the Hering-Breuer stimuli. Inflation proceeds until the inhibitory impulses traveling up the vagi inhibit inspiration and initiate expiration. The important point for us is that all recent work confirms the work of Hering-Breuer that inhibiting afferent impulses are conducted by the vagus to the respiratory center. The question of vagus apnea is then in the last analysis, merely whether these inhibitory impulses can be summated and thus outlast a period of over-ventilation.

In discussing the possibility of a nervous or vagal apnea a great deal has been made of the effects of vagotomy. Some of the older workers

not knowing the rôle of CO_2 in respiration felt that the undoubted difficulty usually experienced in producing apnea after cutting the vagi was sufficient evidence for the existence of vagal apnea. All workers know however that in a few animals apnea is easily produced after vagotomy. Scott (2), Milroy (3) and Eisenhardt (4), using cats and rabbits, all secured apnea after vagotomy. Too much, however, must not be made of this point. The evidence for chemical apnea is overwhelming and neither the presence nor absence of the vagi should interfere with artificial ventilation washing out CO_2 from the alveolar air and blood. Certainly apnea thus produced seems in no way to preclude another possibility, namely, summation of inhibitory influences which come in by way of the vagi. It seems to us that nothing can be settled by the results of vagotomy. Apnea after vagotomy merely shows that chemical apnea can be produced without the vagi, and no one, so far as we know, would care to oppose such a conclusion. On the other hand the unquestioned difficulty of producing apnea by over-ventilation in the dog and rabbit immediately after vagotomy always suggests that the vagi may in some way have aided in the result even though it could be attained without them.

The existence of one thing very seldom in itself excludes the existence of another. Theoretically, the unquestioned fact, since the work of Fredericq, that there is such a thing as chemical apnea does not exclude another form of apnea. So long as the two kinds of apnea, however, can only be produced by the same procedure, namely, over-ventilation, the existence of the second is of course gratuitous. The problem then resolves itself into the production of an apnea by a method which excludes any decrease in the CO_2 of the arterial blood.

The most obvious procedure would be to over-ventilate with an air mixture having a percentage of CO_2 equal to or exceeding that in the alveolar air. Some class experiments on dogs, very poorly controlled though they were, led us to believe that we might succeed in this way.

A study of the literature disclosed that several investigators had carried out or had been credited with having carried out such experiments. Haldane and Lorrain Smith (5) found that distention of the lungs of a rabbit with strong CO_2 arrested respiration even during hyperpnea. Mosso (6) showed that inhalation of a deep breath of hydrogen, but not of CO_2, produced temporary apnea in man.

Haldane and Priestley (7) made on themselves what they considered the "crucial experiment" of ascertaining whether apnea could be produced with air containing only a little less CO_2 than alveolar air. They noted a short apneic pause after a single deep respiration of air, but none after a similar respiration of 5.54 per cent CO_2. Neither did seven forced respirations of 4.93 per cent CO_2 give a pause, but one was very evident after six similar inspirations of room air. While there was no apneic pause after

the CO_2 respirations, the authors' note and their curves show a reduction in respiratory amplitude. Although the authors conclude that they found nothing to suggest that repeated distention of the lungs produced a summated effect on the respiratory center through the vagus nerves, the decreased amplitude succeeding the CO_2 respirations might certainly be so interpreted.

Milroy (3) shortened the apneic pause following ventilation by replacing the air with mixtures rich in CO_2. The Hering-Breuer distention inhibition was studied by Christiansen and Haldane (8) who found that it was not followed by an apnea if the inflation was caused by air containing sufficient CO_2 to prevent a fall in alveolar CO_2 percentage.

The above workers were concerned with chemical apnea and in no case were the distentions repeated often enough to settle the question of inspiratory inhibitory summation. Weil (9) however directed a research directly at this point. In rabbits he produced apnea with from 20 to 60 blasts of air. On adding CO_2 to the ventilating mixture he decreased the lengths of the apneic pauses and found that none could be produced if the CO_2 percentage exceeded a certain value. This value lay between 4.3 and 6.8 per cent of CO_2, and was of course practically the composition of the alveolar air. On the basis of these results Weil decided against the possibility of any other form of apnea than a chemical one. Our results will be seen to be quite the opposite of Weil's. On this fact we can make no comment, further than to note that different species were used by the two workers. It might be that the rabbit's respiratory center shows but little ability to summate vagal influences and that the dog is more favorable for this phenomenon.

EXPERIMENTAL. *1. Apnea with an excess of CO_2.* Dogs, under morphine-ether anesthesia, were used in all the experiments. Artificial inflation of the lungs was produced by an electrically driven pump which forced 318 cc. of air into the tracheal cannula at the rate of 96 times per minute. From 25 to 35 strokes of the pump were used in most experiments. The inlet for the pump could be left open to the air or connected to a large tank containing a mixture of air and any desired percentage of CO_2. The tracheal cannula had an open slit to allow the escape of excess air and to allow the chest to resume in part its previous position between the blasts. The respiratory record was made by a tambour and air transmission from an inflated rubber bag tied directly over the animal's thorax and abdomen.

The essential feature of these experiments being over-ventilation with a mixture of air having a CO_2 percentage above the alveolar, every effort was made to make sure this end was attained. To be sure that the air used for ventilation was richer in CO_2 than the alveolar it was of course necessary to analyze the latter. Alveolar samples were obtained at the

end of expirations by means of a catheter inserted through the tracheal cannula as far as the bifurcation. The air was drawn into a receiving burette over mercury and analyzed in a Haldane apparatus. The difficulty of securing uniform and consistent samples of alveolar air was fully realized. The CO_2 content varies particularly with the degree of anesthesia. In practice we found that the ether could be regulated so that the alveolar air would run from 6 to 7 per cent CO_2 for fairly long periods, and when this condition was reached the inflations were made.

CO_2 was admitted into the large tank in sufficient quantity to make the percentage higher than alveolar. The air was thoroughly stirred with an electric fan to insure the mixture being uniform. The exact CO_2 percentage was obtained with the Haldane.

For fear that the air from the tank might in some way become diluted during its passage through the pump, a sample was drawn during inflation

TABLE 1

Effects of over-ventilation, 30 to 35 blasts, with air richer than alveolar in CO_2

EXPERIMENT NUMBER	ALVEOLAR CO_2, PER CENT	CO_2 IN AIR FROM TRACHEA, PER CENT	LENGTH OF APNEA		pH OF CAROTID BLOOD		APNEA WITH ROOM AIR IN SECONDS
			Number of respirations	Seconds	Before	During apnea	
20	6.4	7.4	9.0	6.5	7.415	7.280	10
21	6.3	7.8	4.5	7.25	7.400	7.263	15
23	6.0	6.4	3.0	4.25	7.389	7.273	29
24	6.7	10.5	4.5	7.5	7.604*	7.265	25
25	6.6	7.1	2.0	5.25	7.491	7.290	18
26	6.6	6.8	7.0	18.00	7.460	7.290	46
27	6.5	6.9	6.0	7.0	7.380	7.290	20

* Sample for this determination was taken during an apnea produced by ventilation with room air.

either directly from the trachea or from the tracheal cannula. This was found to be necessary for usually the valves of the pump exerted a little suction before closing which brought in some room air through the slit in the tracheal cannula.

Finally to avoid all errors in regard to the composition of the air actually delivered, in some experiments the pH of arterial blood from the carotid was determined by the gas chain method before and during a period of ventilation. If the pH were lowered there could be no doubt that we were ventilating with an atmosphere richer in CO_2 than the alveolar air. Although we have many experiments, the technique of which we believe to be above question, a report will be made only of this last series.

In table 1 the data for these experiments are presented. The important fact brought out is that over-ventilation with an atmosphere proved to be richer than alveolar air in CO_2 by analysis of samples taken from the

trachea and by a decrease in the pH of the carotid blood, is followed by a cessation of all respiratory activity. The pause outlasts the periods of rhythmical lung distentions and so is not the same phenomenon studied by Christiansen and Haldane (8). It cannot be due to a block of blood poor in CO_2 reaching the respiratory center, because the blood pH is low. It seems to us therefore that the pause is an apnea in the original sense of the word. In the table we have given the length of the apnea both in seconds and in the number of respirations omitted, calculated from the preceding rate. Figure 1 is the graphic record from experiment 21.

In length the pause following ventilation with increased CO_2 is usually very much shorter than that obtained with room air, although in experiment 26 it was 18 seconds long. So far as cessation of activity in the respiratory center is concerned the inhibitory influence resulting from overventilation is not nearly so effective as that due to lack of CO_2. Although in experiment 24 we secured an apnea on inflation with 10.5 per cent CO_2, the result was not often obtained when the inflating air was more than 1 per cent richer in CO_2 than the alveolar.

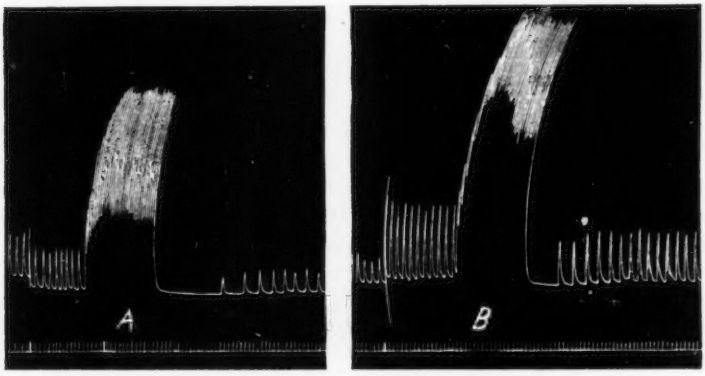


Fig. 1. A: Apnea due to over-ventilation with room air. B: Apnea from over-ventilation with air containing 7.8 per cent CO_2.

So far our experiments actually give no information in regard to the nature of this apnea except that it is not due to lack of CO_2. The well-known Hering-Breuer effect on sustained inflation of the lungs would of course lead one to believe that we were dealing with sensory inhibitory influences transmitted by the vagi. A further series of experiments has given greater probability to this idea.

2. Apnea after vagotomy. Reference has already been made to the well-known fact that apnea can be secured after vagotomy. The fact is equally

well known that in the dog, at least, apnea is produced with great difficulty, if at all, immediately after section of the vago-sympathetics. The failure of the phenomenon does not seem to be due to insufficient ventilation, for although certain blasts of the pump are neutralized by the regularly recurring vagal respiration, the total ventilation is far above normal. The assumption that vagal influences were absolutely necessary for apnea quite naturally grew out of these experiences, although a better knowledge of the blood gases has shown the invalidity of much of the argument.

A series of experiments which we have made, however, seems to prove that impulses were being transmitted by the vagus at each inspiration and that without these, for a time at least, chemical apnea itself was impossible. The experiments consisted of stimulating the central end of one vagus in a vagotomized dog with a very weak tetanizing current during the periods of inflation. The idea was to imitate the conditions that occurred when the nerves were intact. The results have been striking and one such may be seen in figure 2. This animal had been made apneic repeatedly by 25 blasts of room air. After cutting the vago-sympathetics, 50 blasts, as may be seen in figure 2 A, had no effect. The same number of ventilations, however, accompanied with weak tetanic stimulation of the central end of the right vagus at each blast gave an undoubted apnea of 34 seconds, as may be seen in figure 2 B. Figure 2 C shows that the stimuli in themselves were inadequate to stop respiration. Similar demonstrations may be made with considerable regularity and we have many records of them.

Discussion. The fact that a short apnea can be produced by overventilation with an atmosphere rich enough in CO_2 to exceed the alveolar air and sufficient to lower the pH of arterial blood clearly indicates a form of apnea other than the chemical one. There is no evidence that the CO_2 was concentrated enough to paralyze the respiratory center, and the several checks on the analyses of the CO_2 atmosphere and the direct determination of the pH leave no doubt that the CO_2 content of the blood was always above normal.

Knowing that stimulation of a proper strength applied to the central end of the vago-sympathetic inhibits the respiratory center and that a maintained distention of the lungs abolishes the activity of the center, one naturally explains the phenomenon as a vagus apnea. This explanation seems justified by finding that after vagotomy, at a time when ventilation fails to give apnea, the condition may be again produced by ventilation plus synchronous stimulations of the central end of one vagus.

The experiments do not exclude the idea that sensory impulses other than those carried by the vagi may have a regulatory effect on the respiratory center. Boothby and Shamoff (10) have shown such a possibility. Enough of the impulses do run by the vagi however to give the short apnea.

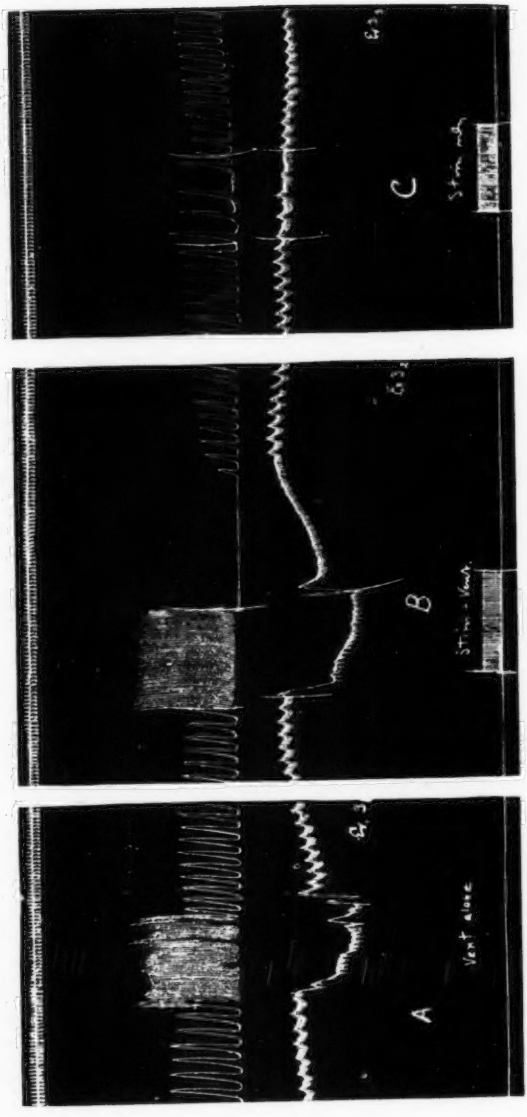


Fig. 2. Upper curves are respiration, middle, arterial blood pressure, and lower, signal. A: Artificial respiration after the vagi were cut. B: Artificial respiration accompanied with weak tetanic stimulation of the central end of the right vagus during each inflation. C: Tetanic stimuli alone, of the same rate, strength and duration.

Since the inhibitory effect outlasts the period of over-ventilation and there is no permanent distention of the lungs, the respiratory center must summate the inhibitory influences. This is sufficiently strong to counteract the stimulating effect of the CO_2, but only for a short time, and apparently only for a concentration of CO_2 not very much greater than the alveolar. Only once in experiment 24, did we secure an apnea with an atmosphere containing as much as 10 per cent. Distention of the lungs with much higher percentages of CO_2 may abolish respiration during the period of distention, as shown by Haldane and Lorrain Smith (5).

Theoretical objections to the conception of vagus apnea have been made on the ground that such a reaction could not possibly be to the advantage of the organism. This objection does not have great weight since practically, as has just been pointed out, a high level of CO_2 seems to make a vagus apnea impossible. No physiological action can however be excluded merely because it does not seem teleological. Inhibition of the heart during asphyxia and high intracranial pressure unquestionably occurs and it is very difficult indeed to explain how this benefits the organism.

In the preliminary experiments the author gratefully acknowledges the help of Margaret Craighill Vickers and Eleanor Leslie.

SUMMARY

1. A short cessation of respiration has been brought about in the dog by over-ventilation with atmosphere richer in CO_2 than the alveolar.

2. Apnea which has become temporarily impossible after section of the vagi has been secured again by applying very weak faradic stimuli to the central end of one vagus synchronously with the inflations.

3. The conclusion has been drawn that the phenomenon was one of true vagal apnea.

BIBLIOGRAPHY

(1) Haldane: Respiration. Yale University Press, 1922.
(2) Scott: Journ. Physiol., 1909, xxxvii, 301.
(3) Milroy: Quart. Journ. Exper. Physiol., 1913, vi, 376.
(4) Eisenhardt: Pflueger's Arch., 1912, cxlvi, 447.
(5) Haldane and Smith: Journ. Path. and Bacteriol., 1892, i, 178.
(6) Mosso: Arch. Ital. de Biol., 1903, xl, 19.
(7) Haldane and Priestley: Journ. Physiol., 1905, xxxii, 257.
(8) Christiansen and Haldane: Journ. Physiol., 1914, xlviii, 272.
(9) Weil: Arch. f. Exper. Path. u. Pharm., 1906, liv, 285.
(10) Boothby and Shamoff: This Journal, 1915, xxxvii, 413.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XVI. The Normal Blood Sugar of Pigeons and Its Relation to Age, Sex, Species and Certain Diseases

OSCAR RIDDLE and HANNAH ELIZABETH HONEYWELL

From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y., and the Department of Physiology, Columbia University

Received for publication September 18, 1923

In earlier studies (1), (2) we have shown that the blood sugar of doves and pigeons markedly increases in close coincidence with each ovulation period. In the two next following studies of this series of papers (3), (4) we have demonstrated two additional factors which involve the blood sugar level on the one hand and reproduction on the other. In still another study (5) observations were made on the behavior of blood sugar values in heredity. In connection with our examination of these several relationships of blood sugar to reproduction and to inheritance it was found advisable—since absolutely homogeneous material for such studies can not be obtained—to undertake a wider survey of the relation of age, sex and certain frequently occurring diseases of pigeons to the blood sugar values shown by them; and with these data available to learn as definitely as possible the normal blood sugar concentration of a number of races, species and genera of pigeons. It is the result of this survey that is presented here.

Though it was not the main purpose of this study to throw light upon current questions concerning the mobilization and metabolism of sugar in the organism we think that some of the results bear upon these problems, since many new data are supplied for a group of species thoroughly adjusted to a high carbohydrate diet.

We may properly urge that the measurements presented are free, or practically free, from some errors which are probably included in much earlier work designed to determine the "normal" concentration of blood sugar in various animal species; for, besides care to avoid previously recognized sources of error or irregularity, three hitherto unrecognized factors which modify the blood sugar have here for the first time been consciously recognized and eliminated. These factors are: proximity to ovulation; close confinement or inactivity, and the onset of cold weather. The present results also offer the unusual opportunity of a comparison of blood sugar values obtained from seven genera belonging to a single order of animals, and of measurements extending from the embryonic period to old age within single species.

METHODS AND MATERIALS. Maclean's micro-method of sugar estimation has been used throughout this study. The technique employed in obtaining blood samples from the heart of adult birds has been earlier described (2), (6). Samples from embryos were obtained by decapitation and collection of the blood in pits cut into paraffin blocks. In this collection care was taken to include in the samples as little as possible of the clear serum which appears on the freshly cut surface of the musculature of the neck. From young birds aged 21 days (one week after hatching) to 3 months the samples were drawn from a wing vein. Though samples from adults were drawn by three different methods (needle-puncture of the heart; from the wing vein; and by cutting the upper beak and allowing the blood to drop into cold paraffin cups) we have observed no consistent difference of sugar content in blood obtained by these different methods. Only very small amounts of blood were taken (0.5 to 2.0 cc. unless unknown hemorrhage into pericardium occurred in cases of heart-puncture) and second or third samples were taken only at long intervals. We have found the wing vein the most practicable and accessible source of the blood. The feathers near the humeral-radial joint are drawn, the skin sponged and dried, the vein punctured with a large curved needle, and the blood allowed to collect in the pit formed by skin anterior to the joint.

The precautions taken to avoid emotional glycemia are recorded elsewhere (2). These include the very rapid collection (in less than one minute) of the sample after taking the bird in hand for this purpose. Speed was particularly sought in the case of birds unaccustomed to handling; and in three typical cases the actual time consumed in obtaining samples was 25, 28 and 42 seconds.

Until caught and removed to small cages for a period of quiet and recovery from fright, all adult birds were permitted to feed freely upon their usual mixed grain ration, since Honeywell (6) has previously shown that the blood sugar of adult pigeons is apparently but little affected by fasting during as much as 48 hours; and further because we found a 19- to 20-hour fasting period to have no measurable effect on 4 common pigeons (179 mgm.), while from 4 ring doves values higher than normal were found (168 mgm.). Since we have found no evidence of an alimentary glycemia in adult pigeons we here record the ration regularly fed to them and kept always before them: for common pigeons (and the two derived varieties): wheat, Kaffir corn, hemp, cracked maize, charcoal, grit, oyster shell, cuttle-fish bone and rock salt; for all other doves: the above ration to which millet and canary seed are added.

Though the birds usually had food in their crops, none was fed during the period of 2 to 5 hours immediately preceding the drawing of the blood samples; during this period, as noted elsewhere, the birds were trans-

ferred from their usual cages, conveniently placed in very small cages for a later blood sampling, and permitted to recover from the fright incident to being caught and transferred. For young birds being fed partially digested food by their parents an interval of fasting is required before the normal value for the adult is obtained. Table 2 gives all our data on this point. We have collected most of the blood samples between 11:00 a.m. and 6:00 p.m., but some samples were collected at earlier and slightly later hours.

The nature of the hybrids used (table 4) has been fully described elsewhere (5), and the common pigeons and ring doves used have also been sufficiently described in the preceding paper of this series (2). The "ataxic" and "scraggly" pigeons are mutational varieties derived from common pigeons. Their characteristic defects probably make them less active as adults than are the common pigeons from which they arose; the feathers of the "scragglies" do not conserve the body heat of the animal to the usual or normal extent. Neither ataxia nor scragginess is recognizable in the embryo, but embryos classified as scragglies are from matings which should yield such embryos almost exclusively. The individuals of the wild species of pigeons used (table 4) in this study were reared in captivity from imported birds. To this there are but two exceptions. The *Spilopelia suratensis* was imported, but in captivity during 3 years, and the *Leucosarcia picata* during 15 years. The exact age of all the other 415 birds used for the present study is known. For these birds a total of 709 sugar determinations is tabulated.

In most cases the birds were killed immediately after or soon after obtaining the last blood samples in order to learn whether they were healthy or diseased. Some of the birds used were, however, too valuable to kill, and this makes it impossible to classify a few of the (younger) groups by sex. All cases of intestinal worms (*Ascaridia maculosa*, Rudolphi) could of course be positively identified; while tuberculosis other than the earliest stages could usually be definitely identified by macroscopic observation at necropsy. Birds bearing both tuberculosis and round worms are classified in the tables as tuberculous. Excepting the 7 tuberculous common pigeons of table 1, none of the birds used was known to be diseased at the time of drawing the blood samples. Nearly all other adult birds were not only sexually mature but gave evidence of freedom from advanced disease by continued reproduction.

Three of the 24 *Turtur orientalis* taken for study (table 3) and killed for examination proved to be partial hermaphrodites—minute testes with left oviducts. One of the hybrids of table 4 had no trace of gonads or of sexual ducts; it is classified as a male (slightly tubercular). It gave a blood sugar value of 150 mgm. per 100 cc. of blood.

Practically all adult birds had been allowed to develop to maturity in a large indoor-outdoor flying space; the common pigeons in a space approximately 25 × 15 × 10 feet, the smaller ring doves in spaces approximately 15 × 5 × 7 feet. After maturity, and to the time of taking the samples, nearly all the adults birds were paired and kept in wire cages 7 × 2 × 6 feet; these were quite open to the sunshine and to fresh air except during cold weather when the sides of the cages were inclosed in glass and heat was supplied. But no data for winter conditions are given here.

All of these sugar determinations were made between March 18 and October 15. Nearly all were made between April 28 and September 15. The sugar values which we call "normal" are therefore obtained during summer. Duplicate determinations which checked to within at least 10 mgm. were obtained in most cases. In the others one to five determinations were made. In the summary of these results we have omitted those determinations which failed to check with other determinations on the same bird, or which were single determinations and fell beyond the limits of checked determinations for the race or species.

PRESENTATION OF DATA. *a. Tuberculosis and Ascaridia.* Seven of the 15 tuberculous birds listed in tables 1 and 3 gave abnormally high values for sugar in the blood. Two gave abnormally low values; 3 gave normal values and 3 others gave values only slightly higher than normal, if the average for the healthy birds of the group be taken as the normal. The 16 tuberculous hybrids of table 4 show a definitely lower average value than that of their 49 healthy associates (160:167 mgm. per 100 cc. of blood). Only 4 of this group had abnormally high sugar values; 3 or 4 had normal values; and 8 had abnormally low values. The details of these data therefore emphasize the tabulated averages in indicating that tuberculous birds should be excluded from others in any attempt to determine the normal blood sugar value in these species and varieties of animals. Our data show that the values obtained from tuberculous birds are usually abnormal, and that these may be either very high or very low. In our material we are unable to associate a high or low blood sugar with any particular type or degree of tuberculosis.

The effect of the presence of Ascaridia (round worms) on the blood sugar is not made wholly clear by our data. This effect seems not to be the same in the generic hybrids of table 4 as in other hybrids; nor is it the same as found for the pure species listed in tables 1 and 3. All of the four groups of Ascaridia-bearing generic hybrids of table 4 show blood sugar values higher than the normal (average of all = 176:167). Among the birds listed in table 3, however, the sugar values obtained from Ascaridia-infested birds seem definitely lower (146:164), and in table 1 they usually seem slightly lower, than the values obtained from healthy birds. But for the groups listed in table 1 this difference is slight and not really con-

TABLE I

The blood sugar values of common pigeons (with two derived varieties, "scragglies" and ataxics) and ring doves classified as to species, age, sex and disease

KIND OF PIGEON		SEX	HEALTHY OR DISEASED	BODY WEIGHT (GRAMS)	NUMBER OF:		AMOUNT OF SUGAR (MGM. PER 100 CC. OF BLOOD)		
					Birds	Sugar determinations	Minimum	Maximum	Average
Common pigeons (Columba domestica)	Adult	Males	Healthy	333	16	53	153	203	181
			Ascaridia	352	7	22	140	199	177
			Tuberculous*	337	3	6	175	245	201
		Females	Healthy	340	6	13	175	220	190
			Ascaridia	330	8	15	158	195	178
			Tuberculous*	299	4	10	178	265	202
		Average	Healthy+Asc.	337	37	103			181
	Young (2.0-2.5 mo.)	Males	Healthy (?)	324	5	10	158	210	188
			Ascaridia	373	3	6	175	185	181
		Females (1.9-2.6 mo.)	Healthy (?)	292	3	6	158	180	171
			Ascaridia	325	5	10	183	193	188
		Average	Healthy+Asc.	334	16	32			183
"Scragglies"	Adult	Males	Healthy (?)	320	5	10	128	180	150
			Ascaridia	351	6	10	140	208	176
			Tuberculous	311	1	1			110
		Females	Healthy	324	3	6	142	145	144
			Ascaridia	328	7	11	130	203	162
		Average	Healthy+Asc.	332	21	37			160
	Young	Sex (?) 2.7 mo.	Healthy (?)	294	2	4	150	160	155
Ataxics	Adult	Males	Healthy (?)	308	2	5	153	198	177
		Females	Healthy (?)	315	2	6	180	185	183
		Average		312	4	11			180
	Young	Sex (?) 2.1-2.5 mo.	Healthy (?)	320	4	7	138	210	184

TABLE 1—*Concluded*

KIND OF PIGEON		SEX	HEALTHY OR DISEASED	(BODY WEIGHT GRAMS)	NUMBER OF:		AMOUNT OF SUGAR (MGM. PER 100 CC. OF BLOOD)		
					Birds	Sugar determinations	Minimum	Maximum	Average
Ring doves (St. risoria and St. alba	Adult	Males	Healthy	165	11†	18	130	178	153
			Ascaridia	172	3	6	128	180	148
			Tuberculous	138	1	2			193
		Females	Healthy	163	11†	18	123	160	149
			Ascaridia	165	7	12	130	160	144
			Tuberculous	160	2	4	135	155	145
		Average	Healthy+Asc.	165	32	54			149
	Young	Sex (?) (1.9-2.2 mo.)	Healthy (?)	143	6	12	135	165	149

*Very advanced stages of tuberculosis.

†Five of these birds were not killed for examination but were probably healthy.

sistent; it would seem that the sugar values obtained from the worm-infested birds of these latter groups may be considered normal.

b. The relation of sex to blood sugar. The data for groups other than generic hybrids may be first examined. If averages obtained from healthy adult birds only be considered (tables 1 and 3), the males of the following groups seem to have slightly higher concentrations of sugar in the blood than have the females. "Scragglies," 150:144; ring doves, 153:149; mourning doves (*Zen. carolinensis*), 262:253. The females average higher in: Common pigeons, 181:190; ataxics, 177:183; *T. orientalis*, 189:192; *Stig. senegalensis*, 174:177. If the Ascaridia-bearing birds be added to the "healthy" birds—a procedure essentially justified for certain races in the preceding section—this larger number of determinations shows a similar approximation of the blood sugar in the two sexes: Scragglies, 164:157; ring doves, 152:147; common pigeons, 180:183. The three hermaphrodites (*T. orientalis*) all gave abnormal sugar values, but all were diseased. From these data we can only conclude that in these groups of birds a measurable difference of blood sugar with reference to sex in general has certainly not been found.

The healthy birds in the large groups of generic hybrids of table 4 rather consistently indicate that the males in the reciprocal crosses have a somewhat higher sugar value than the females (178:166; and 165:159). The birds bearing Ascaridia are, however, not thus consistent (183:175;

and 169:177). In these hybrids it was noted above that the Ascaridia-infested birds of all four groups show higher sugar values than do the healthy birds, though an opposite tendency is elsewhere observed in other kinds of birds thus diseased. This group of generic hybrids was of quite advanced average age (113.4 months) and it is perhaps conceivable that there was more incipient tuberculosis among the females than among the

TABLE 2

Blood sugar values of dove embryos and of young doves after various periods of fasting

KIND OF PIGEON	STAGE, OR AGE IN DAYS (AFTER EGG OF ORIGIN WAS LAID)	NUMBER OF HOURS SINCE (HATCHED) BIRDS PERMITTED TO TAKE FOOD	NUMBER OF:		BODY WEIGHT (GRAMS)	AMOUNT OF SUGAR FOUND		
			Birds	Determinations		Minimum	Maximum	Average
Common pigeons	Full-term (18 da.) embryo		4	4	10.4	146	175	157
	30–36 days	7–10	4	7	216.0	175	183	179
	35–48 days	18–19	9	14	255.0	112	190	161
	45–49 days	Not fasted	3	5	295.0	242	280	256
"Scragglies"	Full-term embryo		2	4	11.2	135	145	140
	29–43 days	9–10	3	6	212.0	128	173	155
Ataxies	39–53 days	Not fasted	3	6	285.0	175	245	208
Ring doves	Full-term (14 da.) embryo		6	6	5.1	120	153	133
	28–44 days	7–15	18	23	110.0	95	180	149
	28–42 days	16–19	21	26	94.0	115	165	143
	38–41 days	Not fasted	6	11	133.0	143	223	164
	21–25 days	Not fasted	6	9	62.0	180	213	191

males. If so, this may account for part or much of the discrepancy, since tuberculous birds of these groups have lower than normal sugar values. It is not possible to decide whether a sex difference does or does not exist in this particular division of our material.

For two additional groups of generic hybrids a much smaller amount of data is available (bottom table 3). In both of these groups the data indicate equal amounts of sugar in the blood of the two sexes (190:192 and 163:164). For most groups of birds, whether hybrid or of pure species, it can therefore be said that our data indicate an equivalence of blood sugar in the two sexes.

A special interest attaches to the figures obtained from the *Spilopelia* × *Streptopelia* cross (table 3) since the number of birds examined is fairly

large, all the birds used are full brothers and sisters, and all were killed in the first year of maturity (aged 6 to 16 months). We shall later publish data showing, within this group, a correlation between sugar concentration on the one hand and thyroid and suprarenal size on the other. The birds (males and non-ovulating females) giving lowest sugar values had larger thyroids and suprarenals; and those giving highest sugars had smaller thyroids and suprarenals. These observations plainly suggest one possible basis for the rather widely varying sugar concentration often found for different individuals of the same race or species.

c. The relation of age to blood sugar. Most of the data for blood sugar at various ages will be found in tables 1 and 2; they refer chiefly to common pigeons and to ring doves which, nevertheless, represent two different families of the order *Columbae*. Some of the young birds used were too valuable to kill and their sex and their freedom from intestinal worms is still unknown. In a previous section it has been noted, however, that neither sex nor the presence of Ascaridia appreciably modifies the blood sugar of common pigeons and ring doves. Since the averages for the adults of these groups include the birds with intestinal worms (very harmful or advanced cases of intestinal worms were not present, since only supposedly healthy and actively reproducing birds of these groups were taken for study) it is quite improbable that our failure to kill and examine these young birds appreciably affects the values listed for them.

Combining the stages given in tables 1 and 2 we find as follows for common pigeons: embryos, 157; 1.1 months, 179; 2.3 months, 183; adult, 181. For "scragglies": embryos, 140; 1.2 months, 155; 2.7 months, 155; adult, 160. For ring doves: embryos, 133, 1.2 months, 149; 2.1 months, 149; adult, 149. These data indicate that the normal amount of sugar in the blood of the adult rather closely approximates to that present at the end of embryonic life and at intervening periods. It is further indicated that the amount of sugar present in the blood of the three kinds of embryos is somewhat less than in the adults of the corresponding species, but that the differences found in the blood of the embryos is as characteristic of the species as is that of the adult. These points can be seen to advantage in figure 1.

Two points in the data just given require further consideration. The first of these is the probability that the blood samples obtained from the decapitated embryos were slightly contaminated with the colorless serum which slowly filters from the freshly cut tissues of the neck. The drops of blood are of course also in momentary contact with at least a part of this cut surface and doubtless carry away traces of it. We have been unable to collect a sufficient quantity of this serum to make a test of its reducing capacity. But it probably has a lower sugar concentration than has the blood, and we suspect that its admixture with the blood sample is the source

of the relatively slight difference observed in the blood of embryo and adult. The second point concerns the amount or degree of fasting necessary in order to obtain a "normal" blood sugar from young birds still being fed partially digested food by the parents. Or, perhaps the real question is, what is the "normal" in such young birds? For the purposes of the list of sugar values given above, and for the construction of figure 1, we have chosen a value (for birds about 1.2 months old) on the basis of reasons which may next be given.

The data of table 2 demonstrate that while being fed by their parents, and during those hours of the day when their "crops" are filled with food, young pigeons show a definite hyperglycemia. Much higher values are then regularly found than is found for the embryo, the adult, or for these same young late at night or before feeding in early morning. After 7 to 10

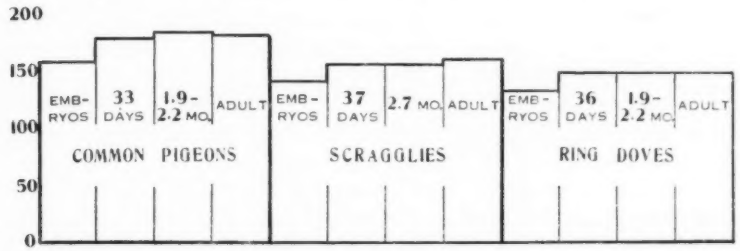


Fig.1. Polygons showing the amount of blood sugar for each of three kinds of pigeons at four different periods of life. The ordinates represent milligrams sugar per 100 cc. of blood.

hours of fasting the young of common pigeons show a sugar value, 179, which is practically equal to that of the adult, 181. After 18 to 19 hours of fasting the sugar value in such young is plainly lower, 161, than that of the normal adult. A fasting period of 18 to 19 hours is longer than the normal interval between the last evening-feeding and the first morning-feeding period in pigeons, i.e., it is longer than any naturally occurring fasting period. It can certainly be said that a fasting period of 7 to 15 hours actually occurs daily in the life of the bird. Similarly, very young "scraggly" offspring showed a sugar value of 155 after 9 to 10 hours fasting as compared with a normal adult value of 160. And finally, the young ring doves fasted during 7 to 15 hours gave a wholly normal sugar value of 149. All of these groups show that a longer period of fasting is accompanied by sugar concentrations lower than normal, and if not fasted at all these groups of young—still being fed partially digested food by parents—all show a sugar concentration higher than is found at any other time in their life history.

TABLE 3

The blood sugar values of various doves and pigeons classified as to species, sex and disease

KIND OF PIGEON	SEX	HEALTHY OR DISEASED	BODY WEIGHT	NUMBER OF:		AMOUNT OF SUGAR (MGM. PER 100 CC. OF BLOOD)		
				Birds	Sugar determinations	Minimum	Maximum	Average
Turtur orientalis	Males	Healthy	226	13	26	160	205	189
		Tuberculous	221	2	2	190	250	220
	Females	Healthy	223	5	9	175	210	192
		Tuberculous	231	1	1			225
	Unknown	Healthy (?)	230	4	8	180	202	191
	Hermaphrodites	Ascaris	218	1	2			155
		Tuberculous	239	2	4	200	205	203
	Average	Healthy	226	22	43			190
Stigmatopelia senegalensis	Males	Healthy (?)	96	3	6	162	195	174
	Females	Healthy (?)	96	2	3	170	185	177
	Unknown	Healthy (?)	90	4	6	150	175	158
	Average	Healthy (?)	94	9	15			168
Zenaidura carolinesis	Males	Healthy (?)	124	1	3	255	270	262
	Females	Healthy	120	1	2	250	255	253
Spil. chinensis	Females	Healthy (?)	170	1	3	160	180	173
Spil. suratensis	Male	Healthy (?)	180	1	3	165	175	170
Leuc. picata	Male	Healthy (?)	420	1	5	180	200	190
♂ Spil. suratensis × ♀ Streptop. hyb.	Males	Healthy	143	9	15	130	185	163
		Ascaridia	149	5	10	125	175	142
		Tuberculous	129	5	8	130	200	154
	Females	Healthy	141	11	23	135	208	164
		Ascaridia	150	5	12	135	172	149
		Tuberculous		2	4	150	183	166
	Average	Healthy	142	20	38			164
Zenaida-Zenaidura hybs.	Males	Healthy (?)	97	2	3	180	200	190
	Females	Healthy (?)	95	2	3	185	200	192

The above data show that the normal concentration of blood sugar is obtained in young common pigeons and in the scragglies after 7 to 10 hours fasting; and that in ring doves this value is obtained after 7 to 15 hours. The fact that hyperglycemia regularly occurs in these very young pigeons during the several hours of each day of the feeding period suggests either that the efficiency of the glycogenic functions is not yet fully established at this stage; or that the absorptive powers of the young bird's intestine—crammed with food already partially digested—are quite out of proportion to the absorptive powers possessed by the bird a month later (see table 1).

The associated fact—that so short a period of fasting as 16 to 19 hours results in a hypoglycemia in these young birds—is equally interesting, since Honeywell (6) has shown that a fasting period of even 48 hours has little effect on the blood sugar of adult common pigeons. Also our more recent tests show that no hypoglycemia results, either in common pigeons or in the ring dove adults, from fasting 19 to 20 hours. This very different result in very young and in adult pigeons is probably associated with the extraordinary rate of growth which is prevalent at this stage in the life of the young birds; but it is clear that an alimentary glycemia is present in the young birds being fed partially digested food, and that in this same stage the blood sugar of the young pigeon is affected by short periods of fasting. For other stages of life there is no evidence that an alimentary glycemia occurs in the pigeon.

On the question of blood sugar concentration in birds of very old age we have the following data: The five youngest (11.1 months) healthy *T. orientalis* (table 3) gave an average of 183; the five oldest (56 months), 188. The 10 youngest (adult) common pigeons (7.5 months) average 177; the 10 oldest (20 months) average 181. The 5 youngest adult "scragglies" (6.6 months) average 164; the five oldest (16.2 months) average 161. The 10 youngest (adult) ring doves (8.4 months) average 151; the 10 oldest (47 months) average 149. The 21 healthy male and female generic hybrids of table 4 which were aged 75 to 100 months gave an average sugar value of 168; while 11 similar hybrids aged 130 to 183 months averaged 171. From these data, and from an examination of the details on which the averages are based as well as from smaller amounts of data obtained from the smaller groups of birds, we find it wholly improbable that very old age in pigeons is normally accompanied by any change in the concentration of sugar in the blood.

It can therefore be said that the data obtained for common pigeons and ring doves at several ages, extending from the unhatched embryo to old age, lead to the conclusion that the normal concentration of reducing substances in the blood normally remains at the same level throughout the whole life cycle. The amount found for the embryos is somewhat less than

that found for all later stages; but the difficulty of obtaining blood samples of embryos uncontaminated with a serum pressed from the cut tissue is probably the explanation of this deficiency.

d. The normal blood sugar of various species and races. Sugar determinations have been made on nine species, two derived races and on three

TABLE 4

Blood sugar values (non-ovulation stages) of a group of generic hybrids classified as to direction of cross, sex, health and disease; average weight of body, suprarenals and thyroids given for all classes

KIND OF HYBRID	SEX	HEALTHY OR DISEASED	NUMBER OF SUGAR DETERMINATIONS	NUMBER OF BIRDS	BLOOD SUGAR (MGM.)	AVERAGE WEIGHT OF		
						Body (grams)	Suprarenals (mgm.)	Thyroids (mgm.)
♂ T. orientalis × ♀ Strep. alba	Males	Healthy	25	14	178	194	16.2	26.7
		Ascaridia	6	3	183	210	16.3	24.5
		Tuberculous	6	3	159	208	19.1	25.0
	Females	Healthy	20	11	166	195	12.8	20.3
		Ascaridia	2	1	175	204	12.3	26.0
		Tuberculous	2	1	185	170	22.6	21.6
♂ Strep. alba × ♀ T. orientalis	Males	Healthy	20	11	165	204	14.6	24.2
		Ascaridia	8	4	169	192	12.8	24.2
		Tuberculous	10	6	154	208	21.2	26.4
	Females	Healthy	21	13	159	189	12.6	24.9
		Ascaridia	12	6	177	180	13.4	22.1
		Tuberculous	12	6	143	181	17.3	47.2
Totals or means	Male	Healthy	45	25	171	199	15.4	25.5
	Female	Healthy	41	24	163	192	12.7	22.6
	♂ and ♀	Healthy	86	49	167	196	14.1	24.0
		Ascaridia	28	14	176	197	13.7	24.2
		Tuberculous	30	16	160	192	20.1	30.1

groups of generic hybrids. For three or four of these groups the number of observations made and of birds used is too small to afford very definite information. Within the nine species seven genera are represented, and these genera belong to two families and four different sub-families of Columbae. The data of tables 1 to 3 make it fairly clear that all except two of the species examined from these genera are characterized by distinct and different concentrations of reducing substances in their blood. A list of

these genera with the adult sugar values found for them, together with the number of birds and of observations placed in parentheses, is given below.

Streptopelia	wt. = 165 grams (32–54);	sugar = 149 mgm.
Stigmatopelia	wt. = 94 grams (9–15);	sugar = 169 mgm.
Spilopelia	wt. = 175 grams (2–6);	sugar = 172 mgm.
Columba	wt. = 337 grams (37–103);	sugar = 181 mgm.
Turtur	wt. = 226 grams (22–43);	sugar = 190 mgm.
Leucosarcia	wt. = 420 grams (1–5);	sugar = 190 mgm.
Zenaidura	wt. = 122 grams (2–5);	sugar = 258 mgm.

The weights given in this list are of course for the species and individuals actually used in the study. It is quite clear that there is no relation between the size of the species studied and the percentage of sugar in the blood.

Discussion and interpretation. Reference should here be made to previous measurements of blood sugar in birds. The nature and extent of this work, together with many points facilitating its consideration, can be most conveniently presented in the form of table 5. The tabulated data make it clear that but little work has been done with the purpose of learning the normal concentration of sugar in the blood of birds. Only four distinct kinds of birds have hitherto been studied and among these there is probably not a single pure or natural species represented. The "fowls" studied by the different observers may have been very different races—probably more different than our common pigeons and the "scragglies" derived from them; and these two groups we have shown to be characterized by two different blood sugar levels. The same would apply to the various studies on "ducks" and "geese." Common pigeons too are mongrels, not a species, and nearly 200 such races exist. The common pigeons used by us are a mixture of "homer" and "carrier," and in some cases the "Jacobin" is also represented in the cross. These birds therefore correspond fairly closely in their origin and composition to the "pigeons" usually available as laboratory animals in this country. In this connection it should be recalled that we have elsewhere (5) shown that blood sugar levels are inherited in crosses.

Table 5 also shows that quite different values have been obtained for the fowl by the various investigators; and the same is also true in the case of the duck. We are tempted to assume that much of these differences is also assignable to different degrees of emotional glycemia and to different periods or degrees of confinement prior to the taking of the samples. In the work of Scott and Honeywell (13) hyperglycemia due to fright was probably successfully avoided; and a comparison of the values obtained for the common pigeon by them with those obtained by us indicates that their birds were not affected by too close confinement. In practically none of the other studies listed is it possible for the reader to learn that either of these two important factors was excluded.

It is further notable that Flemming (12) obtained values (75 mgm.) for ducks, and Marañon and Rosique values for the dove (80 mgm.) which are wholly at variance with all other observations on birds and which are quite similar to values found for mammals. Flemming's several determinations are consistently low; but he makes no note of the wide discrepancy between his own and earlier findings. Unfortunately only the abstract of the work of Marañon and Rosique has been available to us. Unless the low values found in the two above-mentioned studies were the

TABLE 5

A summary of earlier measurements of the blood sugar in birds

BIRD	NUMBER	AMOUNT OF BLOOD SUGAR (MGM. PER 100 CC.)		METHOD USED	OBSERVER
		Range	Average		
Fowl	8	188–250	209	Abeles	Saito and Katayama (7)
Fowl (adult)	4	175–190	183	Bierry-Portier	Giaja (8)
Fowl (2 mo.)	4	200–240	220	Bierry-Portier	Giaja (8)
Fowl	?	? ?	140	?	Bernard (cited from Bang)
Fowl	?	? ?	230	Bierry-Portier	Bierry and Fandard (9)
Duck	7	120–200	160	Abeles	Weintraud (10)
Duck	14	120–180	146	Abeles	Kausch (11)
Duck (fasting)	5	140–160	150	Abeles	Kausch (11)
Duck (sick)	5	130–150	140	Abeles	Kausch (11)
Duck	12	50–98	75	Pflüger-Allihn	Flemming (12)
Geese	6	120–160	138	Abeles	Kausch
Common pigeon	22	160–239	185	Maclean	Scott and Honeywell (13)
Dove ?	?	? ?	80	Bang	Marañon and Rosique (14)

result of prolonged and extreme confinement of the animals used we are wholly unable to interpret them.

Only Giaja (8) has compared the blood sugar of the young and adult of the same race and he made but four observations on each group. He concluded, however, that young fowls—aged about 2 months—have clearly higher sugar values than fowls aged at least one year. The value obtained by him for the adults was, however, considerably less than that obtained by Saito and Katayama (7). The high values obtained by us for one stage of young pigeons we have interpreted as a hyperglycemia resulting from their being fed partially digested food by the parents; there being either failure of full development of the glycogenic function, or an excessively rapid absorptive rate from the intestine, at this stage of growth. This type of feeding does not exist in the fowl: if therefore the young of the fowl should later be proved to have a higher sugar value than that of the adult

it would be made clear that our interpretation of this point is inadequate or wrong.

Kausch (11) states that fasting up to 72 hours does not measurably lower the blood sugar of ducks. Here, however, one would wish to know whether or to what extent emotional glycemia entered into the values found. Honeywell (6) was unable definitely to decide whether 48 hours of inanition had an effect on the blood sugar of 35 common pigeons; the birds used in that study were obtained from dealers and the values found (166 before and 165 after) were lower—both before and after the inanition period—than is now recognized as normal for common pigeons. As earlier noted, this point was further examined by us in tests of the effect of a 20-hour period of fasting and no decrease was found. From these several results, and limiting our statement to the kind of food and method of feeding used by us, we can state as follows: It seems probable that in the pigeon—an animal using a high carbohydrate diet but unlike the rabbit in feeding only by day—the only notable alimentary glycemia which occurs is that during feeding by the parents in the short period of immaturity. This point probably involves a notable difference in one or another aspect of the carbohydrate metabolism of mammals on the one hand and of birds like the pigeon on the other. The alleged greater difficulty of obtaining a pancreatic glycuresis and glycemia in birds may be related to this same difference.

The measurements made on the blood sugar of several wild species of birds, as carried out in the present study with the elimination of many factors known to modify such results, make it quite certain that in at least one large order of birds a very high concentration of reducing substances is normally present in the blood. The different genera and species of Columbae also further show relatively wide differences in normal concentration of sugar in the blood.

SUMMARY

Measurements of the normal concentration of the reducing substances of the blood were made on 415 birds belonging to nine species, two derived races, and three groups of generic hybrids. These species include seven genera and two families of *Columbae*. Most of these species and genera are found to have different and characteristic concentrations of blood sugar. The species examined vary between 149 and 258 mgm. sugar per 100 cc. of blood.

The factors previously known to modify the normal sugar level have been considered, minimized or eliminated from the results; and in addition the data obtained are free from errors arising from three hitherto unrecognized factors—proximity to ovulation, prolonged inactivity or close

confinement, and the onset of cold weather. The nature and condition of the animals used are fully described.

Tuberculous birds usually have abnormal concentrations of sugar in the blood; this concentration may be either high or low. The presence of round worms (*Ascaridia maculosa*) in the intestines does not seem to affect the blood sugar values of all species and hybrids in a similar manner. In reproducing common pigeons and in ring doves the concentration of sugar was unaffected.

Males and non-ovulating females do not show consistent or unquestionable differences in blood sugar.

There is no relation of the size of the races and species studied to the normal concentration of the sugar of the blood.

The blood sugar has been measured in the embryo, in two juvenile stages, in adult stages, and in old age. The values obtained for these several ages are notably similar in any single species or race. The values actually obtained for embryos are slightly lower than for adults, but the apparent difference is probably not real.

In that part of the juvenile period during which the young is being fed partially digested food by the parents, the hours of active food absorption are characterized by values higher than those of the normal adult. In these cases the normal adult values are obtained only after 7 to 15 hours of fasting. It is only during this part of the growth period that an alimentary hyperglycemia has been found in pigeons.

The normally high concentration of the blood sugar of birds, the relative freedom of pigeons from alimentary hyperglycemia (despite the high carbohydrate diet of these species), and the stability of their blood sugar concentration under shorter periods of fasting, all suggest that they differ from mammals in one or another aspect of their carbohydrate metabolism.

BIBLIOGRAPHY

(1) Honeywell and Riddle: Proc. Soc. Exper. Biol. and Med., 1922, xix, 377.
(2) Riddle and Honeywell: This Journal, 1923, lxvi, 340.
(3) Riddle and Honeywell: This Journal, 1924, lxvii, 333.
(4) Riddle and Honeywell: This Journal, 1924, lxvii, 337.
(5) Riddle and Honeywell: Amer. Nat., 1923, lvii, 412.
(6) Honeywell: This Journal, 1921, lxiii, 152.
(7) Saito and Katayama: Zeitschr. f. Physiol. Chem., 1901, xxxii, 231.
(8) Giaja: Compt. Rend. Soc. Biol., 1912, lxxii, 102.
(9) Bierry and Fandard: Compt. Rend. Acad. Sci., 1912, clvii, 1717.
(10) Weintraud: Arch. f. Exper. Path. u. Pharm., 1894, xxxiv, 303.
(11) Kausch: Arch. f. Exper. Path. u. Pharm., 1896, xxxvii, 274.
(12) Flemming: Journ. Physiol., 1913, xlv; ibid., 1919, liii, 236.
(13) Scott and Honeywell: This Journal, 1921, lxiii, 520.
(14) Marañon and Rosique: Bol. soc españ. biol., 1916 (June 10).

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XVII. Blood Sugar and Ovulation under Inactivity or Close Confinement

OSCAR RIDDLE and HANNAH ELIZABETH HONEYWELL

From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y., and the Department of Physiology, Columbia University

Received for publication September 18, 1923

It has long been recognized in the practical breeding of pigeons and of poultry that ovulation ceases soon after reproducing birds are confined in spaces so small as to prevent or greatly restrict the normal movements of the animal. This experience has been so general that data on this point need not be submitted here. The physiological question involved is, what changes in the organism are responsible for, or are associated with, the suppression of ovulation by this confinement and inactivity?

Early in our studies on the blood sugar of pigeons it was thought that birds kept in small cages were probably unsuitable for use in determining the normal blood sugar of a race or species. Lusk (1) had observed on the dog that "prolonged confinement in a cage will, after a time, greatly reduce the basal metabolism though there be no loss in weight. Recovery from this condition is achieved through exercise." This observation suggested to us that metabolic disturbances are involved in the loss of reproductive power by birds kept under these conditions. It therefore seemed advisable to test the effect of such confinement on the concentration of blood sugar. Making this test we should learn: First, whether the suppression of ovulation from these sources is accompanied by changes in carbohydrate metabolism as reflected by the sugar concentration in the blood. Second, whether such inactivity and confinement is a factor modifying the normal blood sugar.

Experimental. Blood samples were obtained by needle-puncture of the heart under precautions described in our preceding studies, and the sugar determined by Maclean's micro-method. In this study two groups of birds were used. Series I was composed of 18 common pigeons which had grown to maturity in a large ($25 \times 15 \times 10$ feet) outdoor-indoor flying space. For two months prior to the drawing of the control blood samples the 18 birds had occupied an "outdoor" space $15 \times 5 \times 7$ feet. After obtaining duplicate sugar determinations for these 18 birds on June 13 to 16, they were divided into lots A and B. Lot A, composed of 8 birds

(taken from the head of the list of first samples drawn) which had given an average sugar value of 181 mgm. per 100 cc. of blood, was used as a first control and therefore left in their former quarters. The remaining 10 birds, lot B, were removed to a very small cage ($3\frac{1}{2} \times 2 \times 2$ feet). This cage was placed in a well-ventilated but only partially lighted basement of the laboratory-annex. The poorer lighting was sought in order to insure less dissension and less activity. This lighting was, however, fully adequate to permit the birds to feed and drink freely. An unwished for but unavoidable factor associated with these basement quarters was a somewhat lower temperature (5° to 15°F.) during the hours of sunshine.

TABLE 1

Summary on the effects of inactivity or confinement in small space on the amount of blood sugar in pigeons

SERIES	GROUP	NUMBER OF BIRDS	MILLIGRAMS OF SUGAR PER 100 CC. OF BLOOD			
			Control (normal activity)	Control		After 3-5 weeks confinement and inactivity
				Cont'd.	After inactivity	
I	A	8	181	181		132
	B	10	175		(173)	119
II	A	3	177	180		
	B	4	178			127
Average			178	181	(173)	125

Before removal to their very limited quarters lot B had an average sugar concentration of 175 mgm. On July 5—after twenty days of close confinement—lot B gave an average blood sugar of 119. Lot A, which had remained in their former quarters again averaged 181. At this time (July 5) the position of the two lots was reversed by the return of lot B to their former quarters, and the subjection of lot A to the small basement cage. Five weeks later (August 12 to 14) lot B, now used as a control, gave an average sugar value of 173; and lot A, now the test animals, gave an average of 132. These data are summarized in table 1.

Series II, composed of 7 additional birds, was begun on July 5. The 3 birds of lot A of this series, then giving an initial sugar value of 177, were left in the larger flying pen. The four birds of lot B, which had just shown a normal average value of 178, were placed (with birds of series I) in the small pen. After 5 weeks lot A had an average sugar concentration of 180; lot B, an average of 127.

At the beginning of the experiment the birds of lot A were aged 9.0 months; those of lot B averaged 9.6 months. At this period the average body weight of lot A was 315 grams; that of B, 324 grams. Lot B neither

lost nor gained weight during the period of close confinement (324 grams at close) though the control birds slightly increased their weight to 330 grams during this same period. During its five weeks of close confinement lot A showed a slight loss in weight—to 310 grams. But the two birds of this group which gained weight during this period showed the same change in sugar values as did those which lost weight. In general, in these experiments the sugar values were about equally depressed in birds which gained weight and in those which lost weight during close confinement.

Other data obtained by us clearly indicate that the somewhat lower temperature of the basement room where the birds were closely confined (June to August) can not be the factor responsible for the fall in sugar concentration. These data (2) showed that the temperature of September—though bringing a greater lowering of temperature from that of June than that involved in the change to basement quarters—is without measurable effect on the blood sugar of common pigeons.

Discussion. Three to five weeks of largely suppressed activity associated with confinement in very close quarters was accompanied by a reduction in the blood sugar of 22 birds from an average of 178 to 125—a reduction from the normal of 29.8 per cent. The reversal of these conditions in lots A and B of series I was promptly accompanied by a reversal of the values found; and return to normal conditions was followed by a return to the normal and previously observed concentration of sugar in the blood. Three additional birds showed an initial normal average of 177 and, as a further control, fully maintained a normal average (180) during the five weeks of inactivity in which their associates (series II) showed a fall in values from 178 to 127.

It is therefore clear that the blood sugar concentrations found in these animals are greatly influenced by confinement; and that, if really normal sugar concentrations are sought in these animals, this factor must be eliminated. There is every probability that these findings apply also to the other laboratory animals commonly used in studies on the blood sugar. We believe that failure to eliminate this factor is in part responsible for the different sugar values reported for the same race of animal by various observers.

In our experiments not a single egg was ovulated under confinement though they were being produced in the period before confinement. This conforms to general breeding experience with birds. It thus becomes clear that in birds closely confined *one* condition accompanying the suppression of their ovulation is a great and rapid reduction of sugar concentration in the blood. This point gains added significance through the similar additional observations made by us: First, that the sugar of the blood normally and regularly *increases* at each ovulation period in the pigeon (3), (4). Second, that a dosage of insulin which twice daily lowers the

blood sugar during 1 to 4 hours to only 60 to 80 mgm. is sufficient to prevent most ovulations in pigeons (5). Third, in the next following paper of this series (2) it has been shown that the onset of cold weather—well known to decrease or suppress ovulation in many birds—also causes fluctuations, certainly involving a temporary decrease, in the sugar of the blood. It is thus made clear that these several conditions which tend to lower the blood sugar concentration tend also to suppress ovulation.

CONCLUSIONS

Close confinement with enforced inactivity produces within three to five weeks a decrease of nearly 30 per cent in the normal blood sugar of pigeons. This fact is doubtless definitely related to Lusk's observation that these same conditions greatly reduce the basal metabolism of the dog.

The hypoglycemia incident to the laboratory or other confinement of animals or of man is probably a widespread source of error or of difference in past and current "normal" blood sugar measurements.

These conditions—close confinement and inactivity—result in the suppression of ovulation in pigeons even though the confined animal makes slight gains in weight.

Several facts suggest that ovulation in birds is normally associated with the capacity of the organism temporarily to increase the blood sugar to a value higher than the normal concentration, and that conditions adverse to this capacity tend to suppress ovulation.

BIBLIOGRAPHY

(1) Lusk: Journ. Biol. Chem., 1915, xx, 555.
(2) Riddle and Honeywell: This Journal, 1924, lxvii, 337.
(3) Honeywell and Riddle: Proc. Soc. Exper. Biol. and Med., 1922, xix, 377.
(4) Riddle and Honeywell: This Journal, 1923, lxvi, 340.
(5) Riddle: Proc. Soc. Exper. Biol. and Med., 1923, xx, 244.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XVIII. Effects of the Onset of Cold Weather on Blood Sugar and Ovulation Rate in Pigeons

OSCAR RIDDLE and HANNAH ELIZABETH HONEYWELL

From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y., and the Department of Physiology, Columbia University

Received for publication September 18, 1923

It has been found that the blood sugar is temporarily decreased in pigeons which are unprotected from the onset of cold weather in late autumn. At the same time and under these same conditions the rate of ovulation is also decreased in these animals. During October, 1922, it was possible to identify the specific date of the first severe autumn weather with a pronounced decrease in both the sugar concentration and the ovulation rate in several kinds of pigeons.

These results make it clear that "normal" concentrations of blood sugar may not be obtained from pigeons similarly and recently subjected to cold; and the same will probably also apply to other birds and to mammals. They further show that a reduced ovulation rate is associated in this instance, as in others previously described by us, with a reduction in the blood sugar. It is also obvious that the diminution of the sugar of the blood under these conditions is an item of interest in connection with the greater heat production, as well as possible changes in basal metabolism, which doubtless occurs in coincidence with it.

Consideration of data. *Effects of cold on blood sugar.* The beginning of this study was not planned as a study of the effects of cold on the blood sugar; these effects were encountered in the pursuit of information on normal sugar values. A summary of the sugar concentrations obtained from various kinds of pigeons at various periods with reference to the onset of cold weather is given in table 1. It will be seen that three of the four kinds of adult pigeons maintained their normal (summer) blood sugar concentrations to October 15. But the birds of these three adult groups, together with corresponding groups of young birds which were examined between October 20 to 29, all gave lower than normal sugar values (the adult ring dove value—148—seems insignificantly reduced). The fourth group—the "scraggly" common pigeons—gave reduced sugar concentrations as early as the end of September. The special case of this imperfectly feathered variety will be considered later.

Our explanation of this apparent reduction of sugar values may next be given. On October 19 and 20 the birds were exposed to a fall of the external temperature to 30° and 33°F. This involved a sudden temperature fall of about 10°F. below the minimum of any preceding day of the autumn; and on both of these days the low temperatures were accompanied by winds of relatively high velocity (mean, 19.3 and 21.0 miles per hour) which added much to the cooling power of the air.

The adult ring doves listed in table 1 do not show a significant effect of the cold weather when considered as a single group. Probably this is because five of the nine birds examined were chosen from the one of our

TABLE 1

Summary on variations of blood sugar during onset of cold weather

KIND OF PIGEON	AMOUNT OF BLOOD SUGAR (MGM. PER 100 CC.)									
	April 28 to September 15		September 21 to October 8		October 13 to 15		October 20 to 20		November 18 to 20	
	No.	Mgm.	No.	Mgm.	No.	Mgm.	No.	Mgm.	No.	Mgm.
Common pigeons	37	181	6	182	6	190	7	175*	3	180
Common pigeons (young)	16	183					8	143	8	185
Scragglies	21	160	16	148	8	140	2	150	3	149
Scragglies (young)	2	155			2	134				
Ataxics	4	180	1	183	1	188	4	150	1	175
Ataxics (young)	4	184	1	155	1	160	2	159		
Ring doves	26	149	6	149	3	149	9	148		
Ring doves (young)	6	149					4	121		

* Four of the seven tests were on "ovulation" stages and should have shown higher than normal sugar concentrations.

five buildings best protected from cold and wind. The blood samples from these five birds were drawn on October 20 and 21. This was before any effects of cold weather were understood, but it was nevertheless thought advisable to use birds least exposed to cold since "normal" sugars were sought and cold weather was an untested factor. The five birds chosen from protected quarters showed slightly more (153 mgm.) than the normal sugar value (149 mgm.); and the four adult ring doves which were taken from more exposed locations showed slightly less (142 mgm.) than normal sugar. The young ring doves all clearly showed lower than normal values (average, 121 mgm.). In reality therefore it may be said that the ring doves, like the common pigeons and ataxics, showed decreased amounts of blood sugar after severe and prolonged exposure to cold. The data

suggest that the effects of cold appeared earlier and were more severe in the young birds than in the adults of the various groups.

The summaries of table 1 give data for kinds or groups of birds at various dates, but measurements made on the same individual at various dates

TABLE 2

Blood sugar tests on individual birds before and after onset of cold weather

KIND	NUMBER OF BIRD	TIME OF TAKING EARLIER SAMPLES	AMOUNT OF BLOOD SUGAR (MGM. PER 100 CC.)		
			From earlier samples	October 20 to 29	November 18 to 20
Adult common pigeons	1	Sept. 22–24	195	180*	
	2	Sept. 24	195	175*	
	3	Oct. 7–8	183	170*	
	4	Oct. 14–15	193	178*	193
	5	Oct. 13–14	198*		168*
	6	Oct. 7–8	183	175	
	7			183	180
	Average		*191*	*177**	*180*
Young common pigeons	8			128	183
	9			128	175
	10			125	185
	11			155	188
	12			130	185
	13			150	183
	14			133	193
	15			153	190
	Average			*138*	*185*
Adult ataxic	1	Oct. 6–7	*185*		*175*
Adult scragglies	1	Sept. 23	175	145	
	2	Sept. 23	145	155	
	3	Oct. 7–14	128		153
	4	Oct. 7–14	138		153
	Average		*147*	*150*	*153*

* Ovulation stages and the blood sugar should have been higher than normal.

are not separately presented there. The data for individuals examined after the onset of cold weather, and also at an earlier or a still later period, are listed in table 2. Inspection of these data will show that they fully support the conclusions drawn from the combined data of table 1.

The "scraggly" pigeons are a mutational variety derived from common pigeons. In these birds all of the epidermal system is affected and none of the feathers are normally formed; the birds cannot fly because the wing

feathers offer too little resistance to the air; also, the loose and deficient feathering is obviously incapable of a normal conservation of body heat, so that birds of this type can survive the winter only when kept in heated buildings. It is significant therefore that the weather of the early autumn period—even as early as the end of September—was sufficient to cause a reduction in the blood sugar of these birds. In this case it is also notable that the reduced sugar concentrations persisted (table 1) during the period of nearly two months covered by the measurements. The earlier decrease of the blood sugar and the longer continuance of this decrease in these scragglies, together with the results from the five ring doves from protected quarters, indicate that the observed decrease is not a purely seasonal fluctuation or annual rhythm unrelated to cold.

It should be pointed out that after October 30 all of the birds were given some protection against cold, and were completely protected against wind, by the replacement of the glass sides of the buildings in which they were confined. It will be noted that three weeks after providing this partial protection from cold the blood sugars of common pigeons and of ataxics had again become apparently normal. The reduced sugar values persisted, however, in the scragglies. No data were obtained for ring doves at this period. We do not feel able to speak confidently concerning the time and conditions necessary for a recovery of normal sugar values in any birds except the young common pigeons. In this case it is clear (table 2) that the normal sugar value was restored from a very low level within a period of three or four weeks. Further definite data concerning the temperatures of the period covered by this study are given in the following section and in figure 1.

Effects of cold on the rate of ovulation. We have attempted to show the fluctuations of ovulation rate in relation to the external temperatures by means of curves in figure 1. The temperature curve is based on the records of the Weather Bureau in New York City (32 miles distant). Those temperatures do not very accurately describe the local temperatures at Cold Spring Harbor where the temperatures were usually lower during this period. All of the very low local temperatures were recorded during this period, and the four arrow-heads on the curve represent these lowest local daily temperatures as registered prior to November 25. These lowest minimum daily temperatures occurred on October 19, November 10, 14 and 24. Our lack of a complete record of local temperatures seems to require the use of the New York City temperatures for purposes of the curve.

The number of eggs laid may here be taken as a sufficiently close measure of the number of ovulations. If the number of eggs laid during 5-day periods, from October 1 to November 24, be plotted in the form of curves, we obtain results indicated in the figure. The two most numerous groups

or kinds of pigeons in our collection are here figured separately. The rate of ovulation in the ring doves—from which larger numbers and more reliable data were obtained—promptly decreased after the two periods of notably low temperatures. The common pigeons show some or much decline in ovulation rate slightly preceding these periods of lowest temperatures, but minimal rates follow both periods of lowest temperatures. Both ring doves and common pigeons show at least partial recovery coincident with the general rise in the temperature curve (October 30 to November 9)—a period also coincident with their partial protection from cold as a result of the enclosure of their quarters.

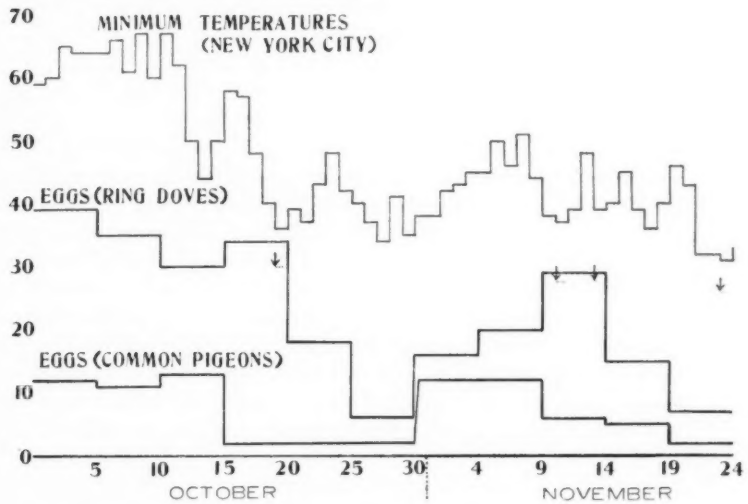


Fig. 1. Showing curves for the ovulation rate of common pigeons and ring doves during the approach of cold weather—October and November, 1922. Days of the months on base line. The ordinates represent the number of eggs produced (ovulations) in 5-day periods, and also temperature (degrees F.). Upper curve, minimum temperatures of New York City. The arrow-heads show local minimum temperatures.

By still other data it can be shown that the season of increasing cold—October 1 to January 1—is characterized by a reduced rate of ovulation in pigeons. The temperature curve of figure 1 indicates a general decline of temperature during October and November, 1922. The records for December are not figured but they show a similar decline. In table 3 we have summarized data on the rate of egg production by 15 individual ring doves for a period which includes all of the year 1922, and therefore the period covered by figure 1, together with the succeeding winter period—

January 1 to April 1. The 15 birds selected for tabulation are those showing highest egg production among a group of 40 birds given opportunity to produce the maximum number of eggs during this period.

The tabulated data clearly show that the period of falling external temperature—October to January—is the period of lowest egg production (107); and that the highest rate (280) is from April 1 to July 1. During this latter period the temperature is already fairly high and is continually rising. That the decline in ovulation rate is not the result of sickness, with permanent suppression of egg production toward the end of the year, is shown by the rise in ovulation rate during the next following 3-month period. The details of the data further show that the period from October 1 to January 1 is also a period during which a high percentage of clutches of "single" instead of "paired" yolks are ovulated.

Discussion. The data of this paper make it clear that pigeons recently exposed to such low temperatures as are normally met with in late autumn usually show abnormal concentrations of reducing substances in the blood, if the concentration found during the summer is taken as the normal. Much the more frequently the blood sugar values are found to be too low after exposure to cold. In most kinds of pigeons the blood sugar apparently returns to the normal after an interval not now definitely known. A notable reduction of sugar values was found 1, 2, 3, 8 and 9 days after a temporary exposure to a temperature of 30°F. and during continued exposure to less severe cold. It is therefore clear that blood sugars obtained from birds recently exposed to cold are not comparable with those not thus exposed; and failure to recognize this factor may introduce serious error into results obtained from pigeons and probably also from many other animals.

Flinn and Scott (7) have studied the immediate effect of higher temperatures during 1 to 6 hours on the blood sugar of dogs. At 20°C. they found during 6 hours a fairly uniform fall in the concentration of the sugar of the blood, but the fall is ascribed to the continued quiet of the dog rather than to the temperature. At 30°, however, this continued decline was scarcely observable beyond the second hour. At 45° and 50°C. during 1 hour, the blood sugar was increased. Hopping (8) found in the alligator a decrease, interpreted as a seasonal variation, in the blood sugar during November–December though the environmental temperature varied but little.

The observed reduction of both blood sugar and ovulation rate as a result of the onset of cold weather supplies an additional instance of this mode of association of the blood sugar and ovulation. It has earlier been shown by Riddle (1) that most ovulations in ring doves are suppressed by the injection twice daily of insulin in doses sufficient to reduce the blood sugar to 62–82 during 1 to 4 hours. We (2) have further shown that close confinement or inactivity during 3 to 5 weeks suppresses ovulation and

markedly lowers the blood sugar. On the other hand, we have made it clear that a temporary increase of the blood sugar occurs in coincidence with the ovulation period in pigeons (3), (4). Parallel with the latter observation Riddle (5), (6) had earlier found a temporary hypertrophy of the suprarenals at the ovulation period. All of these observations suggest that ovulation in birds is normally associated with the capacity of the

TABLE 3

The ovulation rate during the four seasons of the year as found for the 15 ring doves having highest rate of ovulation during the year 1922

NUMBER OF BIRD	NUMBER OF EGGS LAID DURING THE FOUR SEASONS OF THE YEAR									
	January 1 to April 1		April 1 to July 1		July 1 to October 1		October 1 to January 1		January 1 to April 1	
	Total	Singles	Total	Singles	Total	Singles	Total	Singles	Total	Singles
1	22		24		21	1	12		20	
2	19	1	22		21	1	9	1	19	1
3	18		18	2	18		11	1	18	
4	20		22		21	1	5	1	12	4
5	18		18		14		11	1	18	
6	16		18		18		6		14	
7	14		14		22		5	1	14	
8	14		20		18		5	1	14	
9	15	1	19	1	12		10		12	2
10	11	1	19	1	16		7	1	14	
11	10	2	22		15	1	7	1	12	
12	12		18		18		5	1	14	
13	12		18		12		8		12	
14	13	1	16		11	1	4		8	
15	12	2	12		11	1	2		10	2
Totals.....	226	8	280	4	248	6	107	9	211	9
Per cent "singles"...		3.5		2.1		2.4		8.4		4.3

organism to effect a temporary increase of the sugar level beyond the normal, and that conditions which oppose this raising of the sugar level tend to suppress ovulation in pigeons.

The effect of exposure to cold on the blood sugar as here observed by us, and the changes in blood sugar and suprarenal size at ovulation as previously described by us, may next be considered in relation to observations that have been made on the effect of cold on the secretion of adrenin, and to metabolic changes effected by prolonged exposure to cold. Cramer (9) subjected mice to cold and observed that the suprarenals of those mice which best resisted this exposure were well charged with adrenalin, while those that died from such exposure contained little adrenalin. Possibly

the supply of adrenin was here exhausted in the emergency increase of heat production. Very recently Hartman and Hartman (10) have reported that "cooling the skin of an animal (cat) by ice, by immersion in cold water, or by evaporation of water from the skin, causes an increase in output of epinephrin."

In the above observations the time at which the increased adrenin output was found and measured was only a matter of minutes or hours after the application of cold. It seems probable that the increased adrenin output in both of the cases cited above was of too short duration to be really comparable with the periods after cold at which we have found decreased concentrations of blood sugar; that the duration of the hypersecretion continued long beyond the period observed is, however, a possibility. Higher sugar values would ordinarily be expected during the period of an increased adrenin output, but a lower sugar value could result if the rate of sugar combustion were eventually to become disproportionately high. It is further notable that the high blood sugars which we (3), (4) have found associated with ovulation were accompanied by something other than a mere transient secretion—namely, by a hypertrophy lasting several days (5), (6).

Whether the observed reduction of blood sugar and of retarded ovulation rate were accompanied by changes in basal metabolism is, though unanswerable, a question of some importance; for, in one similar and previously described (2) association of suppressed ovulation with reduced blood sugar, there can be little doubt that the metabolism was lowered. In the present case, however, the conditions are those which some investigators consider effective in raising the basal metabolism. On this point it may first be noted that in several communications Boothby and Sandiford (11) have contributed evidence that adrenalin raises the basal metabolism. If this is a fact, and if also the adrenin output under the stimulus of cold is maintained for much longer periods than those already demonstrated by Cramer and the Hartmans and cited above, the presence of an increased metabolism in the birds studied by us is made highly probable. Cannon, McIver and Bliss (12) have very recently suggested that the suprarenals do serve as an important part of the mechanism which prevents the attainment or continuance of too low a percentage of sugar in the blood.

In a report on carefully conducted tests of the effects of out-of-door and indoor conditions upon metabolism Campbell, Hargood-Ash and Hill (13) conclude that "the basal metabolism of the body cells is raised by cool out-of-door conditions, and that shivering is not necessary to raise metabolism. The metabolism is raised by cooling power, not by temperature." Some investigators of the subject, however, do not consider cold, apart from shivering, capable of raising the basal metabolism. One of us has obtained indirect evidence, not easily incorporated here, which is in

best accord with the view that the basal metabolism of pigeons is increased during the approach of cold weather in the autumn. These several considerations suggest—though they do not establish the point—that the suppression of ovulation, along with decreased blood sugar, is sometimes (after close confinement) associated with a lowering of the basal metabolism; and at other times, as in the case of cold weather, with an increased basal metabolism.

SUMMARY

Recent and prolonged exposure to out-of-door air of strong cooling power results in a marked lowering of the concentration of the sugar of the blood of pigeons.

Failure to recognize this effect of cold is or may be a source of error in measurements of the normal sugar level in the blood.

The same temperature conditions which effect a decrease in the blood sugar also diminish the rate of ovulation. At the same time a high percentage of single or "unpaired" ovulations replace normal paired ovulations.

The data supply further evidence that ovulation in birds is normally associated with the capacity of the organism to effect a temporary increase of the blood sugar above its normal concentration, and that conditions which oppose this capacity tend to suppress ovulation.

Some facts suggest that a decrease of both blood sugar and ovulation rate is sometimes associated with a decreased and sometimes with an increased basal metabolism.

BIBLIOGRAPHY

(1) Riddle: Proc. Soc. Exper. Biol. and Med., 1923, xx, 244.
(2) Riddle and Honeywell: This Journal, 1924, lxvii, 333.
(3) Honeywell and Riddle: Proc. Soc. Exper. Biol. and Med., 1922, xix, 337.
(4) Riddle and Honeywell: This Journal, 1923, lxvi, 340.
(5) Riddle: Proc. Soc. Exper. Biol. and Med., 1922, xix, 280.
(6) Riddle: This Journal, 1923, lxvi, 322.
(7) Flinn and Scott: This Journal, 1923, lxvi, 191.
(8) Hopping: This Journal, 1923, lxvi, 145.
(9) Cramer: Journ. Physiol., 1918, lii, 13.
(10) Hartman and Hartman: This Journal, 1923, lxv, 612.
(11) Boothby and Sandiford: This Journal, 1923, lxvi, 93.
(12) Cannon, McIver and Bliss: Boston Med. and Surg. Journ., 1923, clxxxix, 141.
(13) Campbell, Hargood-Ash and Hill: Journ. Physiol., 1921, lv, 259.

THE EFFECT OF THE ORAL ADMINISTRATION OF ALCOHOL UPON THE BLOOD SUGAR OF RABBITS

N. R. BLATHERWICK, L. C. MAXWELL AND M. LOUISA LONG

From The Chemical Laboratory of The Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barbara

Received for publication September 20, 1923

The results presented in this communication were obtained in experiments dealing with the possibility of obtaining absorption of insulin from the gastro-intestinal tract. Since insulin is soluble in dilute alcohol, it seemed worth while to determine whether or not absorption might take place from the stomach when given in alcoholic solution.

The rabbits used were fasted for 18 to 24 hours. Blood samples were taken from the marginal ear veins before and every hour for 4 hours after the solution was given by the stomach tube. The blood sugar was determined by the method of Folin and Wu.

Reference to the table shows that the blood sugar decreased from 14 to 30 per cent of the original values when large amounts of insulin in alcohol were given. The extent of the hypoglycemia indicated that absorption of insulin had occurred. However, when corresponding amounts of alcohol without insulin were given, entirely comparable results were obtained. Four experiments with alcohol alone showed decreases in blood sugar varying from 17 to 42 per cent of the original values. It is perfectly evident that no absorption of insulin had taken place and that the hypoglycemic effect was entirely due to the alcohol. The amounts of alcohol used were not sufficient to produce marked intoxication. That the results obtained were not due to simple dilution of the blood is shown by experiments in which larger volumes of water and of 0.4 per cent sodium carbonate were given. The ingestion of such fluids results in an increase in the concentration of the blood sugar or there is no change.

It had been shown by Fuller (1) that the immediate effect of alcohol is to reduce the hyperglycemia and glycosuria in most cases of mild or moderate diabetes. According to his experiments this effect is most noticeable when the alcohol is substituted for the caloric equivalent of fat, but may also be manifested when the alcohol is added to a given diet. The author found that such effects were usually lacking in diabetes of great severity.

The decrease in the blood sugar of diabetic patients observed by Fuller and the lowering of the blood sugar of rabbits after the ingestion of alcohol reported in this paper are not to be taken as evidence that alcohol will exert a continuous hypoglycemic effect in diabetes. Allen and Wishart (2) and Leclercq (3) have shown that the ultimate effect of alcohol when given to diabetics is to cause a gradual development of hyperglycemia or glycosuria. This result occurs when the caloric intake is raised above the caloric tolerance of the patient.

TABLE 1

Blood sugar of rabbits

RABBIT		BLOOD SUGAR						SUBSTANCE GIVEN BY STOMACH TUBE
Number	Weight	Before	1 hour after	2 hours after	3 hours after	4 hours after	Change	
	kgm.	*mgm. per 100 cc.*					*per cent*	
		133	95	120	110		−28.6	Insulin, 12.5 rabbit units in 100 cc. of 8 per cent alcohol
47	1.47	105	77	111	103	133	−26.6	Insulin, 25 rabbit units in 50 cc. of 4.5 per cent alcohol
28	1.36	121	100	93	95		−23.1	Insulin, 50 rabbit units in 50 cc. of 4.5 per cent alcohol
8	1.47	129	111	111	133	129	−14.0	Insulin, 100 rabbit units in 50 cc. of 4.5 per cent alcohol
50	1.33	118	95	83	91	100	−30.0	Insulin, 60 rabbit units in 50 cc. of 8 per cent alcohol
49	1.98	98	77	89	95		−21.4	70 cc. of 8 per cent alcohol
12	1.87	114	100	95	114		−16.7	70 cc. of 8 per cent alcohol
23	1.76	133	91	77	93		−42.1	66 cc. of 8 per cent alcohol
11	1.53	118	95	103	114		−19.5	50 cc. of 4.5 per cent alcohol
39	1.50	122	124	150			+18.7	Insulin, 42 rabbit units in 130 cc. of ice water
29	2.00	114	114	116	132	121	+15.8	100 cc. of 0.4 per cent sodium carbonate
35	1.93	111	108	103	129	120	−7.2	Insulin, 100 rabbit units in 100 cc. of 0.4 per cent sodium carbonate

SUMMARY

The oral administration of alcohol to rabbits causes marked decreases in the blood sugar. There appeared to be no absorption of insulin when large amounts were given in alcohol, in ice water and in 0.4 per cent sodium carbonate solution.

BIBLIOGRAPHY

(1) Fuller: Journ. Metabol. Research, 1922, i, 609.
(2) Allen and Wishart: Journ. Metabol. Research, 1922, i, 281.
(3) Leclercq: Journ. Metabol. Research, 1922, i, 307.

STUDIES ON THE RELATIVE PHYSIOLOGICAL VALUE OF SPECTRAL LIGHTS

IV. The Visibility of Radiant Energy[1]

HENRY LAURENS

From the Department of Physiology, Yale University

Received for publication September 22, 1923

The basic feature underlying this series of investigations (1) is the consideration given to the distribution of spectral energy as a measure of intensity. The distribution of energy, or the amount of energy emitted at each wave length per unit time (radiant power), is determined by means of a non-selective radiometer with a response directly proportional to the stimulus. It is generally conceded that the most sensitive, as well as the most easily mastered by the non-physicist, means for measuring radiation is the thermopile with auxiliary galvanometer. The spectro-radiometer has been described (1a).

In an earlier paper (1b) mention was made of the fact that the plan in mind at that time included the determination of the visibility of radiation as well as the influence of wave lengths of equal energy on the pupil. The work on the pupil had already been published (1c) and the present contribution sets forth the result of a study of visibility under a variety of conditions. Most of the actual experimental work was done in the years 1919 to 1921 but the preparation of the results for publication has been necessarily postponed on account of the pressure of other work. A companion study of the bleaching of visual purple in strong and weak concentration by wave lengths of equal energy content practically completed during the summer of 1921 has suffered the same fate. This, however, will appear in the near future.

In connection with specific questions pertaining to the present paper the following brief account will give the reader, not particularly familiar with the subject, the fundamental principles involved in and implied by visibility of radiation (2), (3). If a source could be found which emitted equal amounts of energy at all wave lengths a radiometer placed in its spectrum would be affected the same at all wave lengths. If this spectrum were viewed by the human eye there would be perceived a difference in the radiant energy of different wave lengths; the eye distinguishes light

[1] Aided by a grant from the Elizabeth Thompson Science Fund.

of different wave length by its hue. The sensibility of the eye for radiation varies with the wave length and is at a maximum in green light, that is to say, the eye perceives green light most easily. It perceives blue and yellow less easily, violet and red least of all, the sensibility falling off to quite small values at the ends of the spectrum.

This relative sensibility of the eye to the light of different wave lengths, the relation between luminous sensation and radiant energy, has been carefully studied and the values plotted. The curve is the visibility curve of the human eye for an equal energy spectrum and its maximum is at or near 555 mμ for cone vision. The maximum is sometimes quite different for different observers, and the shape of the curves of no two persons is exactly alike. The visibility of radiation at any wave length is the ratio of luminous intensity measured in light units to the intensity of the light measured in energy units. If the relative radiant power (watts) of a source at various wave lengths is multiplied by the relative visibility (lumens per watt) of the human eye at the same wave lengths and the results plotted, a new curve is obtained. This is the luminosity curve and the maximum is near 590 mμ. In other words, the visibility curve is simply the luminosity curve of a source emitting equal amounts of energy at all wave lengths in the visible spectrum,—it is luminosity at constant spectral energy.

Visibility, the ratio of light, apparent brightness, to radiation is determined by measuring the relative spectral energy necessary at each wave length to produce a given luminous sensation or equal sensations of brightness throughout the visible spectrum, the reciprocal of the energy being taken as proportional to the relative visibility. The determination is made by a photometric equation of the given radiation to a standard.

So much has been done upon determining the relation between light and energy another contribution would seem, at first glance, to have little or no value except as being corroboratory and therefore of little interest to anyone but the experimenter himself. But although a great deal of work has been done and the data regarded by those in a position to know as reliable and satisfactory, a need of more extensive statistical determination of visibility is still felt (4) and the continued interest in the subject is indicated by recent announcements of work now in progress (5), (6). It is, moreover, an interesting fact that the results of hardly any two investigators agree; the curves showing greater or less variations particularly in the blue and red, to which great weight is accorded.

My interest in undertaking the determination of this function of vision was not so much in the hope of adding anything to the numerous and accurate observations of physicists and psychologists (among whom should be mentioned, in addition to those already referred to, Ives (7) Nutting (8), Hyde (9), Reeves (10), Bender (11)), who have investigated it, as in the wish

to make a comparative study, to investigate the relative stimulating value of wave lengths in an equal energy spectrum in my own eye for comparison with its other characteristics as well as of those of vision in lower organisms (1b), in connection with the evolution of color vision. It has been pointed out (1a) that in the study of the stimulating effects of the same amount of radiation of various wave lengths for lower organisms, it is necessary to use the same methods and the same general experimental procedure as in determining the relative visibility for man. For the human eye the intercomparison should be made on a fairly large number of different observers, different characteristics being compared on the same group of subjects. But it has not been my good fortune to have at any one time at my disposal more than a small number of observers nor to be able to extend observations on any group to more than one function. Nevertheless there is one subject, myself, who has been observer throughout as well as experimenter, so that eventually it will be possible to compare various characteristics of my own eye with those of lower organisms, and to have results on others as well for other characteristics, and with the same general apparatus or minor modifications of it, as in a study of hue discrimination, or sensibility to wave-length differences (12).

The influence of intensity and its variation is important in the determination of rod, as well as of cone, visibility. Until a short while ago the only usable rod visibility curve which we had was that of König (13) based on calculation of his data by Nutting (8b) and by Krarup (14). Hecht (15) has recently published the results of a careful determination of rod visibility which will be referred to again. Bender (11) investigated rod visibility by eccentric observations. His results are of the first importance in the comparison of central and peripheral vision. The determination of rod and of cone visibility in the same eye has, so far as I recall, only been carried out by König and Bender. My results on cone visibility and rod visibility were referred to in my paper on the relative pupillomotor effects of wave lengths of equal energy. The curves expressing the latter were called motor visibility curves in contrast to those obtained by photometric methods, which may be designated sensory visibility curves.

The importance, or rather the necessity, of working with the equal energy spectrum has been referred to in my previous papers and is probably fully appreciated. But a few words further will not be out of place so as to make the issue clear in the present instance. In a chemical system acted upon by light the extent and progress of the action depends on a number of physical factors of which the principal and all-important one is the amount of energy incident on the system and its spectral distribution. It is impossible to evaluate the relative influence of different wave lengths on a reaction unless their relative intensities are equal or known. This is applicable to vision. It is of course not always necessary

in all visual work that the intensity should be measured in radiometric units,—although the ultimate basis of intensity is radiometric—since photometric measurements must be used in the study of the effect of intensity, as in the sensibility to radiation of constant wave length but of varying intensity. But in studying the influence of wave length, a definite intensity is selected and the wave length varied so as to obtain information concerning the sensibility to the same amounts of radiation of different wave lengths. Such work is possible only when the intensity is radiometrically measured. Visibility is such a problem. The same amount of energy appears differently to the eye according to the wave length; the relation of brightness to physical intensity is a function of the wave length and equal radiant energies do not produce equal brightnesses. As Hayeraft (16) puts it, there is no correspondence between the energies of two lights of different wave length and the effects that they produce upon consciousness.

There are various methods for determining visibility (3), (7), (8). Practically all of the worth-while work on the subject has been done by two of them, both photometric and involving an equation of apparent brightnesses. The first is the method of direct comparison, using an equality of brightness photometer in which two optically contiguous surfaces are separately illuminated at the same time. A modification of this is the so-called cascade or step-by-step method (5), (6), (9). The second is the method of flicker photometry in which the spectral light and a white light are viewed alternately. In the first the two lights are presented at one time in different spaces, in the second in the same space at different times. The two methods do not give identical results and each has its ardent advocates. Our present interest does not extend to picking out the rather small differences in the results of the two methods or of according greater value to the results obtained by the one or by the other. By some the flicker method is said to give a broader and flatter curve (7), (9). Others point to opposite results (3). On the whole the majority of investigators feel that the results obtained with the flicker method have a greater certainty, precision and reproducibility, and that the true brightness is specified by it. For the details of the arguments the reader is referred to papers by Coblentz and Emerson (3), Ives (7), Troland (17), Crittenden and collaborators (18). The case against the flicker method is ably presented by Ferree and Rand (19). Although not primarily interested in the question as to whether the one or the other was methodologically more "correct" we have made determinations by both and with the same instrument. In this connection it may be noted that Kingsbury (20) has devised an attachment for the Lummer-Brodhun contrast photometer so that it may be used for both direct comparison and flicker photometry. The instrument we used is described by Bechstein (21). A lens and aperture system

is rotated by means of a motor. The lights to be photometered are reflected by two faces of a plaster of paris block which meet at a right angle so that the visual field is filled alternately and for equal times with the light from the two surfaces. These latter can be turned through 180° to eliminate slight differences in reflectivity. They were covered at intervals by magnesium oxide by holding them over burning magnesium ribbon. The size of field was two degrees obtained by using a diaphragm with appropriate sized aperture. The eye slit was such that its image thrown on the eye by the eye piece was 2.5 × 0.5 mm. When making determinations by the method of direct comparison, the rotating system was turned by hand until the line of contact between the two surfaces bisected the visual field as a vertical diameter. For a fixation point a glass rod drawn out to a diameter of about 1 mm. and the end ground at an angle of 45° was employed. Dim red light was passed down the rod and scattering at the ground end gave a small dim red source. This method was obtained from Abney and Watson (22).

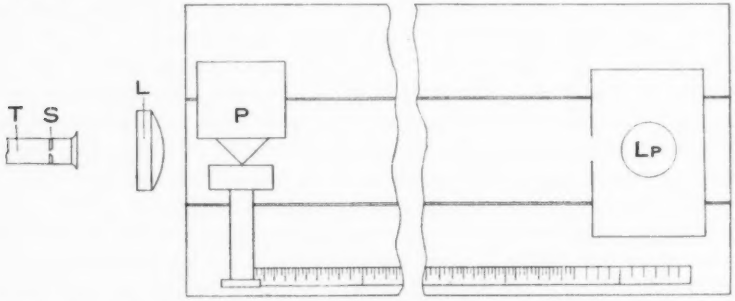


Fig. 1. *S*, slit in telescope, *T*, of spectrometer. *L*, lens delivering parallel rays. *P*, photometer showing two reflecting surfaces meeting at a right angle. *Lp*, source of standard white light.

The distribution of energy in the source has been measured directly at the eye slit by some investigators; by others computed on the basis of the color temperature of the source. We measured it, thus obtaining the energy of the stimulus as applied to the eye. As described in our first paper, the method of work has been to use equal energy, so that the energy of the wave lengths throughout any one series of measurements is constant. That is to employ such a procedure that the results, or their reciprocals, represent the values for an equal energy spectrum. This procedure was again followed in the present work, but only in part of it. It may be designated as the first method. By it the results are specified in terms of the photometric value of spectral lights which are equal in energy. For a given intensity the telescope of the spectrometer (fig. 1) was placed at a cer-

tain distance (which was kept constant) from the photometer which remained stationary. The source of spectral light (not shown in the figure) was placed at the distance found, by previous measurement, to deliver a certain unit amount of energy at the given wave length. The photometer was set for an equality of brightness determination, and the standard lamp then moved until the spectral light and the white light appeared equally bright. Three determinations were thus made and the average of these taken. This being done, a flicker determination was made, the rotating system started, the speed of rotation being altered by variable resistance until color flicker disappeared at which speed it was kept by an assistant who watched for changes in current. After color flicker disappears there is left brightness flicker; this was caused to disappear by changing the distance between the standard lamp and the photometer.

The speed of rotation was increased to, but not beyond, the point at which the color flicker disappeared. This speed gives maximum sensibility, the results being more similar to those obtained with the equality of brightness method. The speed is different for different observers, different intensities and different wave lengths. At low illumination the speed is low, and the ends of the spectrum require a higher speed than the middle, owing to the greater difference between the color of the spectral light and that of the standard. Furthermore the lower the speed the higher the sensibility and therefore the sensibility at the ends of the spectrum is lower than it is in the middle.

In many cases after the equality of brightness set had been made there was no, or very little, brightness flicker after the color flicker had vanished after adjusting the speed of rotation. Some of the determinations were made at minimal flicker, one subject using this method throughout. Another wave length was then selected, the spectral light source placed at such a distance that the energy was equal to the unit and the process repeated and so on through the spectrum. The measure of relative visibility was obtained by taking the reciprocal of the photometric values in meter candles (since the energy was the same for all wave lengths), making the wave length of maximal efficiency equal to 100 and rating the other wave lengths in percentages of this. By this method two complete determinations of spectral visibility, at high (cone) and two at low (rod, gray) visibility were made. It is an admirably satisfactory method, but it does not permit the specification of the light in photometric units, since for each wave length, when the energy is the same, the photometric value changes. In order to be able to specify the intensity in meter candles it is necessary to make the visibility determinations in terms of a constant intensity of white light, that of the spectral lights being varied to match it in brightness. The relative visibility is then computed in terms of the distribution of energy, using the curve representing this, and the relative luminosity

TABLE 1

Relative visibility

WAVE LENGTHS	EQUAL ENERGY RECIPROCAL OF METER-CANDLES		25 M.C.	10 M.C.	2.5 M.C.	1 M.C.	CORRECTED FOR MACULAR ABSORPTION		25 M.C. 5° FROM CENTER	25 M.C. 10° FROM CENTER	5 M.C. 20° FROM CENTER	10 M.C. 30° FROM CENTER	ROD VISIBILITY	
													Equal energy reciprocal of meter-candles	0.2 M.C.
	Flicker. 6 subjects	Equality of brightness. 3 subjects	Flicker. 6 subjects	Flicker. 2 subjects. Equality of brightness. 4 subjects	Flicker. 4 subjects	Equality of brightness. 2 subjects	Not reduced	Reduced	Equality of brightness. 1 subject	Equality of brightness. 1 subject	Equality of brightness. 1 subject	Equality of brightness. 1 subject	Equality of brightness. 2 subjects.	Equality of brightness. 2 subjects.
mμ	○	□	●	■	▲	×		△	+	⊘	↑	↑	↑	↑
400	0.5		0.7			0.9								
410	1.9		1.0	1.4	1.6	1.8	2.1	1.9			2.2		4.1	4.9
420	2.6	1.8	2.0	1.7	1.5	1.9	3.0	2.9	3.6	2.4	3.8	6.1	5.7	6.0
430	3.2	2.2	2.7	2.8	2.0	2.9				3.8	6.0	8.9	9.7	9.2
440	3.5	2.8	3.6	2.8	2.3	3.9	4.8	4.7	4.6	6.8	9.2	14.2	15.1	14.3
450	4.1	3.5	4.2	3.8	4.1	5.1				8.1	13.8	20.8	21.6	22.3
460	5.7	6.1	6.2	6.4	5.5	6.2	9.1	8.8	8.0	12.3	21.5	29.8	29.8	31.5
470	8.9	9.7	10.2	8.8	8.9	10.1			12.0	17.4	34.7	52.2	52.3	52.1
480	13.1	14.6	15.2	14.7	13.1	16.2	21.2	20.6	22.8	24.3	51.7	73.6	74.8	72.6
490	20.3	23.7	24.2	19.6	21.2	25.8			29.3	34.0	75.3	85.3	87.7	88.1
500	32.6	35.0	33.7	31.5	33.7	35.2	44.9	43.7	46.2	53.6	90.8	92.0	93.6	96.8
502.5											92.7	96.4	97.1	98.0
505											93.1	98.9	98.2	99.1
507.5											96.2	99.8	99.0	100
510	49.7	52.5	51.0	47.8	50.0	52.6	64.1	61.1	60.0	70.6	97.1	99.8	100	100
512.5											99.0	100	99.4	98.7
515											100	99.0	97.2	97.5
517.5											98.7			

520	68.2	74.7	65.7	63.2	69.5	71.0	75.5	73.2	75.9	88.2	96.4	96.5	95.8	93.5
530	85.0	87.1	86.0	81.2	85.4	88.0	90.2	87.5	87.3	98.1	91.5	88.7	85.1	85.2
540	94.5	96.3	95.8	94.7	95.2		99.8	96.8	96.9	100	77.7	70.6	65.0	66.1
545	98.2	98.1					101.7	98.6		99.2				
550	99.2	98.9	97.8	99.5	99.1	100	102.4	99.3	98.9	98.2	58.2	48.2	47.6	47.7
552.5	99.7	99.2	98.9	99.8	100	99.3	102.8	99.7	99.4					
555	100	99.8	99.3	100	99.8	98.8	103.1	100	100	96.6				
557.5	99.4	100	99.8	100	99.5	97.9	102.9	99.8	100					
560	98.9	99.6	100	99.5	98.4	97.1	102.6	99.5	97.5	92.7	47.4	38.1	34.6	34.9
570	95.7	95.1	97.2	94.2	94.2	94.7			94.8	82.9	32.7	26.3	27.3	27.8
580	86.0	88.1	89.9	87.1	86.2	85.9	89.1	86.4	83.2	73.0	27.5	20.3	18.1	17.6
590	75.0	78.2	77.3	81.1	78.4	77.6			78.0	65.9	18.0	14.2	12.8	12.0
600	64.2	67.1	65.2	64.3	62.4	61.8	65.9	63.9	68.5	54.2	14.0	9.6	7.9	8.1
610	52.0	53.6	50.1	53.7	47.5	44.2			48.9	46.4	10.2	7.5	6.4	7.0
620	39.6	36.3	39.9	40.2	36.1	29.6	39.4	38.2	33.2	35.2	6.3	3.7	3.8	4.5
630	27.5	26.0	27.2	28.8	20.3	15.9			27.2	26.1		2.0	1.5	2.8
640	18.8	17.9	18.1	18.5	10.4	7.6	18.6	18.0	15.0	17.8	0.27	0.6	0.9	1.0
650	10.4	8.9	11.2	11.8	5.1	3.8			10.8	11.6	0.23			
660	6.1	5.7	6.1	6.9	2.8	1.5	6.3	6.1	6.1	7.1				
670	3.5	3.1	3.2	3.1										
680	1.3	2.0	2.4	1.9										
690	0.9	1.4	1.8											
700	0.2	0.8	0.7											

values of the spectral light in terms of the distance of the source of spectral light from the collimator slit. This may be called the second method.

There are no differences in the relative results obtained by the two methods (see the table and fig. 2), except those due to errors of observation and those within the limits of variation obtained by different previous investigators. By the first method one obtains just as sure a measure of the stimulating efficiency of the wave length as by the second, where the intensity of the standard lamp is kept constant and that of the colored light changed until equality is reached. In the first method the measurement of efficiency is in terms of the meter candles of white light (photometric value of stimuli in M.C.'s) which must be supplied at each wave length in order that this should be equal to the wave length in question. In the second method the efficiency is expressed in terms of the relative energy of the colored lights (radiometric value of the stimuli in relative units) which must be supplied in order that they should appear equal to the white illumination at a certain number of M.C.'s (the relative amount of energy to produce a given luminous sensation).

This latter method is generally regarded as being more accurate in principle. But both give results which are in terms of the amount of energy which must be expended to produce equal results or to bring about the same amount of change. The first method is adequately justified by a consideration of what visibility represents. As above outlined it is the ratio of luminous intensity to energy intensity. By this method the energy is of constant value at all wave lengths. Visibility at any wave length is therefore equal to the reciprocal of the luminosity in M.C.'s.

For the sources of standard white light 10 watt (8.3 c.p.) and 50 watt (38 c.p.) vacuum tungsten lamps were used. These were photometered, after seasoning, against a standard. A 1000 watt tungsten was used as the source of spectral light. Its energy distribution through the spectrum was determined at the beginning and a number of times during the course of the observation and again at the end. The collimator slit was kept at 0.25 mm., that of the telescope at 0.5 mm.

The results are shown in the table and figure 2. Their actual description will be brief. Three series of three determinations each were made under each condition specified by the heading of each column. First as to cone visibility at various intensities: the results obtained by the first method as above outlined are given in columns 2 and 3 for the flicker and equality of brightness methods respectively and the curves indicated by the corresponding symbols at the head of the column. The results for the various subjects are averaged together after having been reduced to equal areas and assigning the maximum wave length a value of 100. The wave lengths of maximal influence are 555 mμ and 557.5 mμ respectively. I shall not enter upon a discussion of the differences between the results

obtained by the method of direct comparison and that of flicker. They varied of course, but none of the subjects were consistent, nor was the magnitude of the variation great enough, on the average, to be disturbing. This was perhaps due to the small (2°) field. Three complete series were obtained on each subject at three separate sittings. Each determination was the average of three sets at each wave length at each sitting.

The influence of varying intensity (25, 10, 2.5, 1 and 0.2 meter candles) is shown in columns 4, 5, 6, 7 and 15 and the values plotted in figure 2. It will be noted that the shift (Purkinje) in the wave length of maximal effect and in the relative effectiveness of the wave lengths on the short and

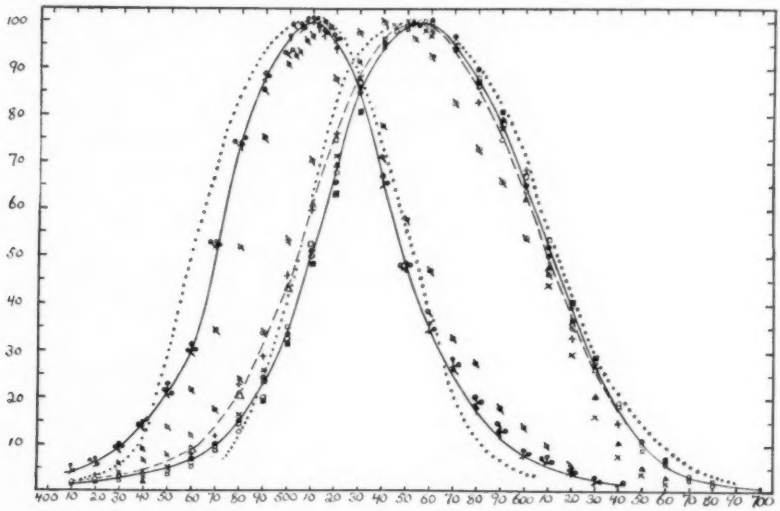


Fig. 2. Curves of relative visibility. The ordinates represent relative visibility, the abscissae wave-lengths in mμ. The symbols correspond to those at the heads of the columns in the table. The pupil curves are shown in dotted lines.

long sides of the maximum is very slight until rod (achromatic or gray) vision is reached at about 0.2 M.C. At 2.5 and 1 M.C. the maximal wave length was shifted slightly blue-wards, the curves becoming slightly sharper at the peak, the short wave lengths increased slightly in relative effect while the long wave lengths decreased, and the red end was shortened (see columns 6 and 7). The actual values for the stimulating effect were naturally lower for the lower intensities, but when reduced on the basis of the maximal wave length being equal to 100, the curves agree quite closely with those at high intensities. We found the flicker method below 2.5 M.C. very inconvenient, even though adaptation and fixation were carefully attended to. At 1 M.C. the results showed such great variation

that the method was abandoned, and records made by direct comparison alone. Others have used the flicker method at low intensity and have obtained concordant results with it; we regret our inability to make use of it for rod vision. We also found that the flicker method was much less convenient than direct comparison in the investigation of peripheral (rod) vision, taken up below.

There can be no question but that the reason why the Purkinje shift has been obtained by others for intensities above the cone threshold (i.e., with intensities of light such that the wave lengths appeared colored) is that in their experiments, owing either to the large field or to deviation of gaze when small fields were used, the image was formed on portions of the retina including rods as well as cones. By limiting the field to 2° we did not actually limit the field to the fovea, or the region where the Purkinje effect is known not to take place (23), (24), (25), but apparently rods, or non-foveal cones, were not stimulated in sufficient numbers or in sufficient intensity to interfere with the results of stimulating the central cones. At the low intensities strict attention was paid to adaptation so that the foveal cones should be at maximal sensibility (26) and every precaution taken against fatigue. Keeping the stimulus on the center of the retina was only done, we are sure, by the use of the fixation point referred to above, and the constant movements of the head and eye (15 to 30 seconds) which shift the image on the retina were not great enough to bring paracentral elements into action.

Although there seems no doubt but that the Purkinje phenomenon does not occur in the fovea although this undergoes extensive adaptation (26), whether it occurs in the entire rod-free area or not would still from the results seem to be a question. The relatively increased sensitiveness for the short wave lengths in dim lights, which is sometimes called the cause of the Purkinje phenomenon is a characteristic of vision in the dark adapted eye, which kind of vision (achromatic) is mediated solely, or chiefly, by the rods.

The Purkinje phenomenon may also be demonstrated by dark adaptation alone (1c) and it can be shown to be due to the action of the rods. In ordinary "mixed" vision, that is, with large fields and moderate and high intensities so that both rods and cones are being stimulated, there is a double influence, one due to the rods and one to the cones. Ordinarily the rod influence is not evident owing to the greater sensibility of the cones. But by dark adaptation this can be brought out with bright light though it is a fleeting phenomenon owing to the quick fatigue of the rods. But if as well as dark adaptation there is a diminution in the intensity of the light, then, if fixation be not provided for, or if the field be large, the curve expressing the relative results of stimulation will indicate the gradual shift from cone to rod vision, the gray vision of the rods adding its influence

to the apparent brightness mediated by the cones and, owing to the greater sensibility of the rods to the shorter wave lengths, the curve will shift bluewards. The amount of shift indicates the relative action of the rods to the combined action of the rods and cones. It is possible that some of the variations in visibility curves are due to the presence in the fovea and in the supposedly rod-free area of rods or of structures with rod-like functions (22). Ives explains his reversed Purkinje shift when using the flicker method (he obtained the usual shift with direct comparison) in contrast with Haycraft's (16) demonstration of the usual shift when using the method of critical frequency on the basis that in his work cones alone were being stimulated. This is as may be. Troland (24) reports that with a 1° field it is not possible to demonstrate any difference between the two methods when the intensity is diminished and believes that measurements of cone visibility may be made at any absolute intensity down to the threshold, provided a foveal field is used. Ives himself (p. 854) says that he later used a 2° field because he had found that the changes in the luminosity curve due to changes in the illumination are less with the small field. Bender (11) found practically no evidence of the Purkinje effect using a 2° field. This matter will be referred to again when discussing peripheral vision.

The high intensity visibility curves are subject to a correction in order to refer the stimulus to the retina. This is due to the selective absorption in the blue and violet by the ocular media and particularly the pigment of the macula lutea. This was referred to in our paper on the pupillar reaction. The loss by reflection at the various boundary surfaces of the eye should also be corrected for if there were any way to determine the amount of the correction since the loss is greater for short wave lengths than it is for long. Abney and Festing (27) determined the relative luminosity of spectral lights in the yellow spot, outside of the yellow spot at ten degrees from the center, and in the fovea alone. They demonstrated an increase in luminosity of the blue and green as far as 526 mμ to the outer portion of the retina, but found the outer part of the retina less sensitive than the central part to the less refrangible rays. The fovea centralis was found to be more sensitive to the red rays than the macula and in general less sensitive to the green and blue, which is surprising on account of the described lack of the yellow pigment in the fovea. The influence of the macula pigment has been studied by several and reviews of the general situation are given by Parsons (28, p. 40), Krarup (14, p. 11) and vonKries (29, p. 341). Wave lengths longer than 590 mμ are not influenced and the absorption is slight for wave lengths longer than 525 mμ. The amount of absorption varies greatly in different cases (1:0.3 for blue; 1:0.5 for green light) so that results based on any curve expressing its influence are at best doubtful approximations. Priest (30) regards the correction as very

inaccurate for frequencies greater than six hundred trillions per second, 500 mμ. The "selective absorption" of the crystalline lens is also likely to render the results of any single individual slightly different from those of others.

To obtain further information concerning the influence of the selective transmission of the pigment in the yellow spot, visibility was determined by direct comparison at an intensity of 25 M.C. at 5° and 10° from the center of my retina. The results are given in columns 10 and 11 of the table and plotted in figure 2. In addition the correction for the relative transmission of the ocular media (24) of the average eye has been applied to the average values of relative visibility as given in columns 2, 3, 4 and 5 of the table, see columns 8 and 9 and curve in dashed lines.

In the first place, my curve at 5° is very similar to that at the center when the latter is corrected for differential absorption. The pigment probably extends further from the center than this, its greatest extent being given as 1 to 3 mm., subtending 4 to 12°, the long diameter being horizontal. But it is probably very dilute at 5° from the center. With a 2° field and central fixation a considerable portion of the field stimulated (the actual fovea) will not exhibit the yellow spot effect if there is no yellow pigment in the fovea. The influence of the fovea then would be to further shift the curve toward the red end than would the macular effect alone. From Abney and Festing's results (see their figure 35) in the fovea alone it would appear that the shift of the curve to the red side as compared with that 10° from the center was partly due to the increased sensitivity at the fovea to the longer wave lengths and a corresponding decrease to the short, when the fovea and the surrounding macula were together stimulated. A considerable portion of the so-called macular effect may be therefore due to the bringing into action of cones with relatively lower sensitivity to longer wave lengths as well as of rods. The curve at 10° besides showing a sensitivity about one-quarter lower than the center indicates more of a shift both in the position of the maximal wave length and of the entire curve to the short side. This is considered as due to the simultaneous stimulation of rods and cones, the stimulation of the rods adding brilliancy (owing to the gray effect) to the wave lengths shorter than the maximum, and subtracting it, so to speak, from those longer than the maximum. It will be noted that the shift on the long side is less than on the short side showing that the rods are relatively less and less (or the cones relatively more and more) stimulated as the wave lengths become longer. This is due to three reasons: *1*, the rods are relatively few; *2*, the sensibility of the rods decreases as compared with that of the cones as the wave length increases; and *3*, on account of the relatively greater selective absorption by the yellow pigment of the blue and green rays than of the yellow, orange and red, the central curve is not so much shifted on the long side as on the short.

This study of peripheral vision at 5° and 10° introduces us to rod visibility, which was studied by the method of direct comparison at low intensity and by eccentric observation (at 20° and 30° to the temporal side) at moderate intensity, the spectrum, however, being colorless. Dark adaptation was attended to. The paracentral values are found in columns 12 and 13 of the table and in figure 2. Our second paper (1b) called attention to the fact that it seemed as if our knowledge concerning the comparison between peripheral vision, central vision, scotopic (dark adapted eye, rod) vision, and color blind vision was in need of revision or reinvestigation. As a result of considerable work, most of it by vonKries or his pupils (28), (29, p. 348), (31), it may be said that the peripheral luminosity curve of the light-adapted eye is different from that of the rods (dark-adapted eye, dim light) and approximates the foveal luminosity curve (light-adapted eye, bright light). The curve of the totally color blind at all intensities is similar to that of the rods (after dark adaptation and in dim light). In dark adaptation and with low intensity, vision is carried out through the rods alone. As the intensity is increased the cone threshold is reached and exceeded and vision is carried out by both rods and cones. At high intensity vision is carried out chiefly or wholly by cones but it is uncertain as to whether the rod effects are added to the cone effects or whether they are abolished. Peripheral vision is central vision (cone vision) diminished in sensibility, while rod vision is a different kind of vision. The visibility of the peripheral cones is the same as that of the foveal cones although the periphery is color blind. Now it seems clear that peripheral vision at high intensity and with the eye in the light adapted state, although colorless, can be nothing more than cone vision, the sensitivity of the rods being low, and therefore show but slight modification from that of central vision. But if the eye be dark adapted, although bright light is used, the rods have a chance to be stimulated. Bender (11) with a 2° field and with the same intensity that he used for central vision by the flicker method has obtained at 20° and 30° eccentric observation curves of relative visibility which approximate closely those of the color blind eye, that is rod vision. All of the other work, the reports of which I have seen, seems, as far as can be made out, because the conditions are not specified, to have been carried out on the light-adapted eye and with large fields and therefore involving mixed vision; with one exception. Siebeck (32) investigated spectral luminosity with a field of 2′.1 at 1°.5 from the center with the light reduced to such an intensity that the spectrum was colorless (minimal field brightness). He found that the curve expressing this relative, paracentral, subchromatic threshold spectral brightness was similar to that of the light-adapted periphery and quite different from that of twilight vision. This seems to indicate that the central, but nonfoveal cones, behave in a similar manner when dark adapted and stimulated by

dim light to the peripheral cones when light adapted and stimulated by bright light, the sensations being colorless in both.

Bender (11), on the other hand, found that colorless relative visibility at 20° and 30°, after dark adaptation although using bright light, was similar to that of two totally color-blind persons. Coblentz and Emerson (3) are led to conclude from this that color perception in total color blindness is similar to that of normal foveal color vision under very low illumination and to rod, or peripheral vision, under high illumination. By normal foveal color vision at low illumination they must mean rod vision, particularly as they state that this visibility curve has a maximum between 490 mμ and 530 mμ.

When the average cone visibility curve is corrected for the influence of differential absorption a curve is obtained which is very similar to that of my own eye at 5° from the center. The 10° curve shows more of a shift to the shorter waves, owing to the fact that more rods, although relatively few, are thus stimulated. The 20° and 30° curves demonstrate that the effect on the rods is much greater than that on the cones, and the 30° curve is a typical rod visibility curve.

The eccentric determinations must be made quickly else the values shift owing to the rapid loss of function on the part of the rods, as indicated by the disappearance of the bright gray dazzle and the appearance of color. A few determinations showed that values at such times were very similar to the central at high intensity. In other words, the values shifted from those of rods to those of cones. A few eccentric determinations were made with the flicker method. After having made the central set using an intensity of 5 M.C. the eye was dark adapted and then the dim red spot fixated so that the image was 20° from the center. The spectral light under these conditions appeared either brighter or darker (according to wave length) than the standard giving rise to a brightness flicker which, however, disappeared as the effects of dark adaptation wore off.

From all that we know of the duplex mechanism it seems perfectly natural that the peripheral vision of the light adapted eye should have more of the characteristics of cone (i.e., central) than of rod vision, although the two kinds of elements are both being stimulated. But when the eye is dark adapted the resulting increase in sensitivity of the rods would presume a vision more rod-like than cone, and such is the case as is seen from my and Bender's work on peripheral vision.

Quite similar results, differing only in degree, were observed on the pupil. I consider that all of these results offer a further or additional substantiation of the Duplicity Theory in that peripheral vision under conditions such that the rods are stimulated, namely, dark adaptation and moderate intensities, shows that the peripheral rods have a function in determining the character or quality of the sensation.

The last point is the determination of rod visibility at low illumination (colorless but bright), namely, 0.2 M.C. This was done for two subjects by both methods. The wave length of maximal effect is 510 mμ, or very close to it (columns 14 and 15). Note the similarity to the 30° curve (column 13).

Hecht (15) points out that his average rod curve shows, when shifted 48 mμ, complete identity with the average cone curve published by Hyde (9). It was considered of sufficient interest to see how the cone and rod curves of my eye determined by the same method would show up by such a comparison. The average of 21 distances between the rod and uncorrected cone curve is 49 mμ (extremes 37 and 64 mμ); between the rod and corrected cone curve 45 mμ (extremes 26 and 63 mμ). The curves therefore would not exactly coincide if one were shifted. But I am not prepared to say that Hecht is incorrect when he says that the two curves, one at high intensity, the other at low, are really the same curve in two different positions. It is evident, however, that the rod curve is steeper and narrower than the cone curve. Something of the same sort of difference has been found in the curves expressing the spectral bleaching values of visual purple solutions as contrasted with those more dilute. Weigert (33) has indicated, though indirectly and by analogy, that visual purple may exist in the cones in very dilute solution as contrasted with its relative concentration in the rods.

One point further, although it is a minor one. We have not been able to substantiate the influence of the correction for macular absorption that Hecht obtains. He used the values given by Sachs which he states to be far from satisfactory. I have added the pupillar curves (1c) to the figure. Their close correspondence with those of the average visibility curve is apparent, so that the correction of the two curves for macular absorption is quite similar. Hecht states that the curve is shifted becoming more symmetrical, so that the maximum is moved from 554 to 540 mμ. I have wondered whether this should rather be 550 instead of 540 mμ. Coblentz (3) finds that the correction shifts the maximum from 557.6 to 556 mμ. The maximum of my average curve is shifted from 557.5 to 555.0 mμ.

The visibility curve has been considered as indicative of a distinction between the brightness and the color of chromatic stimuli. Troland (24), (34) believes that, among other things, the symmetry of the visibility curve points to an independence between the mechanisms subserving brilliance and chroma, luminosity being identified with whiteness. It has, however, been demonstrated in this laboratory (12) that differential fatigue reduces these two aspects of a chromatic stimulus together. In other words, the shape of the visibility curve is altered.

SUMMARY

1. The visibility of radiation, or the relation between light and radiant energy, has been determined for the cones at various absolute intensities; for the more central rods at low illumination; and for the peripheral rods at moderate illumination. The measurements were made by the photometric methods of direct comparison and of flicker.

2. The average cone visibility curve for six subjects is similar to those which have been previously published, with a maximum at 555 $m\mu$ when corrected for the selective transmission of the eye media. It was determined by two methods, *a*, by using the reciprocals of the photometric value of lights equal in energy, and *b*, by using the reciprocal of the energy required to produce a given luminous sensation.

3. The cone visibility curve was determined at intensities of 25, 10, 2.5 and 1 meter candles, always employing a 2° field. The shift (Purkinje) in the relative sensibility to long and short wave lengths was not observed because, by sharp fixation, the stimulus was practically confined to the foveal cones.

4. The rod visibility curve at low, colorless, intensity is of the same general shape, though steeper and narrower, as the cone curve, but its maximum is at 510 $m\mu$, 45 $m\mu$ nearer the blue end of the spectrum.

5. The visibility curve at 5° from the center is very similar to the central curve when this has been corrected for the selective transmission of the ocular media. The curve at 10° shows a greater shift to the blue end of the spectrum owing to the combined stimulation of rods and cones, the curve at 20° approximates that of rod (colorless) visibility at low intensity and that at 30° is practically identical with it.

6. The eye was dark adapted for the visibility determinations at 20° and 30° and intensities of 5 and 10 M.C. respectively were used. Peripheral visibility with a small field (2°) at high intensity in the dark-adapted eye is thus similar to rod visibility, (dim light, dark-adapted eye) while in the conditions of day vision, peripheral visibility is similar to typical central (cone) visibility.

BIBLIOGRAPHY

(1a) Laurens and Hooker: This Journal, 1917, xliv, 504. (1b) Laurens and Hooker: Journ. Exper. Zoöl., 1920, xxx, 345. (1c) Laurens: This Journal, 1923, lxiv, 97.

(2) Gibson and McNickolas: Bull. Bur. of Standards, 1919, Tech. paper 119, 1.

(3) Coblentz and Emerson: Bull. Bur. Standards, 1917, Sci. paper 303, 167. Coblentz: Bull. Bur. Standards, 1920, xv, 639.

(4) Priest: Bull. Bur. Standards, 1921, Sci. paper 417, 231.

(5) Gibson and Tyndall: Journ. Opt. Soc. Amer., 1923, vii, 68.

(6) Priest: Journ. Opt. Soc. Amer., 1923, vii, 99.

(7) Ives: Philosoph. Mag., 1912, 6th Ser., xxiv, 149, 352, 744, 845, 853; Journ. Franklin Inst., 1919, clxxxviii, 217; Journ. Opt. Soc. Amer., 1923, vii, 363.

(8a) NUTTING: Bull. Bur. Standards, 1908, v, 261. (8b) NUTTING: Ibid., 1911, vii, 235. (8c) NUTTING: Phil. Mag., 1915, 6th ser., xxix, 301. (8d) NUTTING: Journ. Opt. Soc. Amer., 1920, iv, 55.
(9) HYDE ET AL.: Astrophys. Journ., 1915, xlii, 285; 1918, xlviii, 65.
(10) REEVES: Trans. Illum. Eng. Soc., 1918, xiii, 101.
(11) BENDER: Ann. d. Physik., 1914, xlv, 105; Zeitschr. f. Sinnesphysiol., 1916, l, 1.
(12) LAURENS AND HAMILTON: This Journal, 1923, lxv, 547, 563.
(13) KOENIG AND DIETERICI: Ges. Abhandl., 1893, 144.
(14) KRARUP: Physisch-Ophthalmologische Grenzprobleme. Leipzig, 1900.
(15) HECHT AND WILLIAMS: Journ. Gen. Physiol., 1922, v, 1.
(16) HAYCRAFT: Journ. Physiol., 1897, xxi, 126.
(17) TROLAND: Journ. Exper. Psychol., 1917, ii, 1.
(18) CRITTENDEN AND RICHTMYER: Bull. Bur. Standards, 1918, xiv, 87. Journ. Opt. Soc. Amer., 1920, iv, 371. CRITTENDEN AND SKOGLAND: Journ. Opt. Soc. Amer., 1921, v, 366.
(19) FERREE AND RAND: Psychol. Rev., 1915, xxii, 110; Trans. Illum. Engin. Soc., 1922, 1.
(20) KINGSBURY: Journ. Franklin Inst., 1915, clxxx, 215.
(21) BECHSTEIN: Zeitschr. f. Instrumentenkunde, 1905, xxv, 45.
(22) ABNEY AND WATSON: Phil. Trans. Roy. Soc., 1916, ccxvi A, 91.
(23) v. KRIES AND NAGEL: Zeitschr. f. Psychol. u. Physiol. d. Sinnesorgane, 1900, xxiii, 167.
(24) TROLAND: Trans. Illum. Eng. Soc., 1916, xi, 947.
(25) KOHLRAUSCH: Pflüger's Arch., 1922, clcvi, 113.
(26) HECHT: Journ. Gen. Physiol., 1921, iv, 113.
(27) ABNEY AND FESTING: Phil. Trans. Roy. Soc. London, 1893, clxxxiii A, 531.
(28) PARSONS: An introduction to the study of color vision, 1915.
(29) HELMHOLTZ: Handb. d. physiol. optik, 1911, ii, 341.
(30) PRIEST: Phys. Rev., 1918, xi, 498.
(31) PEDDIE: Colour vision, 1922.
(32) SIEBECK: Zeitschr. f. Psychol. u. Physiol. d. Sinnesorgane, 1907, xli, 89.
(33) WEIGERT: Pflüger's Arch., 1921, cxc, 177; Zeitschr. f. Electrochem., 1921, xxvii, 481; Zeitschr. f. physikalische Chem., 1922, c, 537.
(34) TROLAND: Journ. Opt. Soc. Amer., 1922, vi, 3.

HEAT REGULATION AND WATER EXCHANGE

I. The Effects of Hot and Cold Baths upon Blood Concentration and Brain Volume in Dogs[1]

HENRY G. BARBOUR

From the Department of Pharmacology, McGill University, and the Department of Pharmacology, Yale University

Received for publication September 24, 1923

While investigating in collaboration with Doctors Baretz (1), Freedman (2), Herrmann (3) and Howard (4), the relation of the water content of the blood to fever, and especially to the action of antipyretic agents, it became evident that great significance must attach to the shifting of water in the heat regulation processes of the normal body.

It was therefore determined to study in dogs the behavior of water in the body in the normal reactions to altered environmental temperatures.

This undertaking was begun by determining the respective effects of cold and hot baths upon the water content of the blood. While this ground work was being laid it was found feasible to study simultaneously the changes in the brain volume.

The latter were considered important not only because of their possible bearing upon the control of heat regulation by the central nervous system, but also because it is conceivable that the brain may serve as one of the storehouses for water leaving the blood.[2]

The conception of blood dilution as a response to external heat accords with a number of older observations upon changes in the hemoglobin per cent, red cell count and viscosity of the blood. These in a general way have all been observed to decrease under the influence of hot baths, and increase with the cold. The results of a number of such investigations will be found in a review by Matthes (5). Such results have not, however, been obtained with constancy, and in the case of heat probably the most widespread impression today is that which conceives of a hot environment as depleting the blood water as a result of sweating, panting, etc. (6).

As regards changes in brain volume, the experiments of Müller and Veiel (7), Strasburger (8), Leonard Hill (9) and others appear to indicate that a cold environment is always associated with an increased brain volume,

[1] The expenses of this research were defrayed from a grant from the Elizabeth Thompson Science Fund.

[2] In this connection suggestive work has been performed by Weed and McKibben who have shown that the brain of the cat will rapidly swell or shrink as the tonicity of the blood is altered by water or saline injections. This Journal, 1919, xlviii, 512.

while a diminution sometimes preceded by temporary increase is seen in the case of heat. Hill showed that the rôle of the blood pressure in this connection was not a permanent one.

To throw further light upon the rôle of the central nervous system, a number of our experiments were done under the influence of anesthetics which, of course, tend to remove this central control.

SELECTION OF A METHOD FOR DETERMINING CHANGES IN THE WATER CONTENT OF THE BLOOD. The existence of no satisfactory method for making repeated determinations of either the total volume or the water content of the blood is generally admitted. Two types of procedure have been followed. First, the introduction into the blood of some foreign substance which diffuses away at a very slow rate, (variations of the dye method introduced by Keith, Rowntree and Geraghty (10)). Variability in the rate of mixing of the injected substance with the blood, and in the excretion factor, detract from the reliability of this type of experiment; furthermore one can never assume that the injected substance itself is wholly inert. In Lamson's (11) recent critical work some of the difficulties of the problem are indicated.

Better procedures for computing the relative water content of the blood are those in which normal blood constituents are measured. In particular, determination of the plasma protein content has been found of value by some. Unfortunately, however, the blood circulation is not everywhere in contact with semipermeable membranes of such character as to retain the protein, and Kestner and others (13) have shown that one of the very stimuli with which we are here concerned, namely, heat, appears to shift protein as well as water from the tissues to the blood. The best criteria therefore are those which depend upon the least soluble and most constant elements of the blood, namely, the corpuscles. The red cell count, the hemoglobin estimation and the percentage weight of the total solids are most commonly employed in such determinations. Of these, the last mentioned, while requiring slightly more time for determination, has in our hands always yielded satisfactory results. The red cell content of the blood upon which this method largely depends is admittedly subject to continual variation under ordinary living conditions. When, however, the factors of diet and changes in environment are eliminated, the hour-to-hour variations in the red cell count, total solids, etc., become insignificant.

In the interpretation of results obtained by the latter type of method one has to choose between the water content of the blood as the sole significant factor, and the possibility that the solid constituents themselves have actually changed in amount enough to affect materially the picture. The assumption of a rapid migration of erythrocytes into, or out of the blood, has sometimes had to be discarded in favor of the more probable theory, that it is largely the fluid content which has altered. This must be especially true of rapid changes, as for example, a 2 or 3 per cent increase in the total blood solids within one hour. The objection that some of the

red cells while not entering or leaving the blood are forced into or out of a temporary condition of stasis, is more difficult to meet.

But in our heat-regulation experiments we may fortunately dispense with the conception that we need to determine with certainty changes either in the *total* blood volume, or even in the relative amount of water in the *entire* circulation; for we require only a definite picture of the relative water content of that part of the blood which for the time being is circulating actively. This is provided by measuring the total blood solids. If the *actively circulating blood* presents a high water content we may be sure that the dissipation of heat from the body is favored, while a diminution in the relative amount of actively circulating water with its consequent increase in osmotic pressure and viscosity of the blood must present the reverse effect.

Limits of variation of the total blood solids in fasted dogs. The following protocols are introduced to indicate the limits of the variations which occur in the total blood solids of dogs which have been fasted for sixteen or more hours except for water, which of course is withheld during the experiment.

I. Dog 48. Irish terrier, male

TIME	WEIGHT	RECTAL TEMPERATURE	BLOOD SOLIDS	HEMOGLOBIN RELATIVE
	kilos	*°C.*	*per cent*	*per cent*
January 2/20				
5:20 p.m.	14.7	39.5		
January 3/20				
11:00 a.m.	14.4	39.4	20.2	104
12:00		38.9	20.1	96
1:00 p.m.		38.7	19.7	96
2:00		38.9	19.8	104
3:00		38.9	19.4	95
4:00		39.1	20.0	97
5:00		39.0	19.9	95

II. Dog 49. Mongrel hound

Fasted December 28 and 29

TIME	WEIGHT	RECTAL TEMPERATURE	BLOOD SOLIDS	HEMOGLOBIN RELATIVE
	kilos	*°C.*	*per cent*	*per cent*
December 30/19	14.6			
10:00 a.m.		38.9	22.6	104
11:00		38.8	22.3	95
14.9 cc. vaccine filtrate				
12:00		38.4	22.4	96
1:00 p.m.		38.8	21.9	91
2:00		39.3	22.3	87
3:00		39.1	22.2	93
4:00		38.8	21.8	90

III. Dog 51. Black and yellow male

Fasted January 8 and 9

TIME	WEIGHT	RECTAL TEMPERATURE	BLOOD SOLIDS	HEMOGLOBIN RELATIVE
	kilos	*°C.*	*per cent*	*per cent*
January 10, 20	10½			
9:00 a.m.		38.2		
10:45		38.3	20.7	97
12:45 p.m.		38.2	21.1	103
21 grams dextrose in 420 cc. H_2O given by hypodermoclysis. Began at 1:40, ended at 2:25.				
2:45		38.3	21.7	105
3:45		38.7	21.4	106
4:45		38.4	21.6	106
5:45		38.9	22.0	84

IV. Dog 34. Black and white male

TIME	WEIGHT	RECTAL TEMPERATURE	BLOOD SOLIDS	HEMOGLOBIN RELATIVE
	kilos	*°C.*	*per cent*	*per cent*
December 29, 19	7.3			
9:45 a.m.		38.7	18.7	100
10:45		38.7	18.3	100
77.75 grams dextrose in 200 cc. H_2O per os				
11:45		38.6	19.7	109
12:45 p.m.		38.5	19.5	103
1:45		38.5	19.5	112
2:45		38.5	19.5	115
3:45		38.9	19.3	107
5:30		38.6		

The first experiment cited exhibits the results obtained every six hours in a normal untreated dog. In the second dog the injection of vaccine filtrate was made as a check against the concentrated coli vaccine which was being used at the time, and since negative results were obtained, serves to illustrate further the value of the blood solids method. In the last two of these illustrative protocols the administration of 5 per cent dextrose, respectively, subcutaneously and by mouth, resulted within an hour in raising the blood solids to a higher level, at which was again maintained a remarkable degree of constancy. In all of these experiments the hemoglobin (Cohen and Smith) readings, which are included, show less constancy.

It will be seen from these illustrations that under proper conditions of diet and environment a variation of over 1 per cent in the total blood

solids over a period of six hours should not be seen; while the greatest hour-to-hour change should not exceed 0.5 per cent.

In the acute heat-regulation experiments about to be reported the changes in total blood solids have greatly exceeded these limits, and are therefore believed to represent fairly the results of the various procedures. The above cited experiments were performed with the collaboration of Dr. A. J. Howard preliminary to work already reported on the antipyretic effects of intravenous injections of dextrose in "coli fever" (4).

Experimental procedures. Dogs of 6 to 10 kilos, with medium length hair were used, and were immersed up to the neck in saline baths in a galvanized iron pail of such size that the animal could just sit comfortably within. The experiments were usually brief and no attempt was made to keep constant the bath temperature, which was simply recorded at the beginning and end of each period of immersion. In other experiments the cold stimulus was a strong current of air at ordinary room temperature blown upon a thoroughly wet animal. In the anesthesia experiments heat was supplied by electric pads, and cold by sprinkling with ice water together with the use of the blower. All rectal temperatures readings were taken at a depth of 8 cm.

The changes in blood concentration were studied by measuring the total solids of samples of about 1 cc. each, taken freely flowing from the marginal ear vein into a tared pair of watch glasses. Each blood sample was weighed immediately and then dried to a constant weight in an oven at about 100°C.

Changes in brain volume were determined first by observation, and more extensively by the use of a simple cranial plethysmograph. The latter consisted of a recording tambour connected with rubber tubing to a brass tube which had been screwed into a 1 cm. opening in the sub-temporal region of the skull under ether anesthesia on some previous day.

In the first experiments the skull was simply trephined, the underlying dura being slit in a number of directions, thus exposing to view the cortex with the pial vessels. The animals were placed suddenly in one of the baths and the results of short periods of immersion upon the exposed brain surface were noted by direct observation.

The dogs all enjoyed excellent health and almost never showed signs of discomfort or made disturbances of any sort.

Results. *Changes in brain volume:* Immersion of a normal dog in a cold (6° to 10°C.) bath gave the following results: The pial vessels blanched rapidly while the surface of the brain within a minute was seen to approach the cranium from which it had hitherto been slightly separated, and before long to bulge slightly through the aperture.

Immersion in a hot (about 40°C.) bath or return to room air from a cold bath if the immersion had not been too long, usually gave opposite results,

namely, shrinking of the brain away from the cranium (leaving room for the passage of a small probe in any direction) associated with flushing of the vessels.

The reaction to heat, however, was not always of this character, the typical effect being sometimes preceded by a reaction similar to that produced by cooling. The following protocols illustrate the results obtained by direct observation of the exposed brain. It will be noted that during recovery from anesthesia the response to the hot bath was more typical than that to the cold.

October 7, 1920. Dog 1. Coach fox terrier; 6 kilos.

10:00 a.m. Morphine sulphate, 5 mgm. per kilo.

10:30 Etherized and trephined in right temporal region. Dura incised.

11:05 Rectal temperature 36.1°C.

11:30 Animal still anesthetized but becoming restless. Immersed in saline bath at 6°C. for 3 or 4 minutes. Brain bulged slightly on immersion, followed at once by shrinking.

12:00 Forty degrees Centigrade bath for a few minutes produced definite shrinking of the brain, 6°C. bath produced little change. Upon repeating the 40°C. bath the brain receded considerably more, the sulci becoming wider.

12:10 After having been out in the room air for about 5 minutes and shivering, the brain surface is observed to be again in contact with the dura on one side. The animal is now partly awake.

2:35 p.m. Rectal temperature 36.7°C. Dog well out of ether.

2:40–3:00 The following observations were made. Brief 9°C. bath, brain bulges. Brief 40°C. bath, little change. Brief 6°C. bath, brain bulges. Removal from this bath to room temperature (19°C.) brain flattens to the level of dura. Brief 40°C. bath, no definite change.

4:30 Ten degrees Centigrade bath, brain bulges. Forty degrees Centigrade bath, no definite change.

October 11, 1920. Dog 2. Tan mongrel terrier; about 10 kilos.

10:00 a.m. Skull trephined beneath right temporal muscle, under morphine and ether, dura incised.

4:27 p.m. Lively. Immersed in saline bath (0.9 per cent) at 40.7°C. for a few minutes. Brain shrinks away from cranium. Pial vessels flushed.

4:40 Changed to bath at 9°C. Brain surface immediately becomes pale and approaches cranium, soon bulging slightly through aperture.

4:45 Taken out of cold bath into room air. Some further bulging was noted.

4:50 In bath at 39°C. brain shrinks, soon leaving considerable space within the cranium, surface again becoming reddened.

5:00 Cold bath repeated with same results as before. Brain continued to bulge for some time after removal to room air.

October 26, 1920. Dog 5. Mongrel; 8.75 kilos. Morphine ether anesthesia. Trephined in temporal region. Trephined opening enlarged to about 3 cm. Dura incised and reflected.

October 27, 1920.

10:10 a.m. Animal appears normal. Rectal temperature 39.5°C. Eight degrees Centigrade bath. Brain bulges gradually. Pial vessels paler (?).

10:32 Out of bath. Brain shrinks slowly.

10:52 a.m.	Into hot bath about 41°C.
11:10	Slight shrinking of brain, vessels much injected. Into cold (10°C.) bath.
11:11	Bulging of brain prompt.
11:15	Vessels now somewhat paler.
11:17	Brain close against cranium. Into warm bath. Brain bulges more.
11:30	Brain bulges less. Animal still shivers. Vessels injected.
11:45	Brain still bulges. Out of bath. Shivers more and brain protrudes decidedly more, being now elevated 1 or 2 mm. beyond outer surface of cranium.
	Rectal temperature 39.8°C.
12:00	Rectal temperature 39.4°C. Brain bulges slightly less.

In experiments upon other dogs a recording tambour was connected with the cranial cavity as described above. The results obtained in dog 3 are illustrated by the accompanying plethysmographic tracings, figures 1 and 2

The effects of the hot bath are seen in figure 1. Immersion at 40°C. gave a marked fall of the tambour, while return to room air after twenty-two minutes resulted at once in an increase in volume.

The cold bath (8°C.) on the other hand produced immediately an increase in brain volume which after five or ten minutes exhibited a further extensive rise. Removal after twenty-five minutes to a room temperature of 17°C. resulted in a corresponding decrease to about the normal level.

In another dog similar typical responses to the cold stimulus and its removal were first obtained (Dog 4). Immersion in the hot bath was made when the animal's rectal temperature was below 34°C. and the cranial volume increased markedly for the first few minutes, showing a reversal of the effect of heat under a condition of extremely low body temperature. Only after about twelve minutes did the brain volume begin to diminish when it fell rapidly under the influence of heating until the pre-immersion level was reached and passed at about the twentieth minute. Thus the typical response to heat was eventually obtained. Forty minutes after removal from the bath and at a time when the animal's temperature was now normal, a return to the hot bath (40°C.) resulted at once in a marked decrease in plethysmographic volume.

In interpreting as actual alterations in brain bulk the changes thus recorded one is supported not only by the direct observations in which the contour of the brain surface was actually seen to alter, but also by the fact that the pial vessels react in the opposite direction from the tambour changes. The increase, therefore, of the contents of the cranial cavity under the influence of the cold stimulus cannot be explained on the hypothesis that cerebral blood flow is augmented.

Effects on the water content of blood: In the experiments upon the two dogs last described, numerous determinations were made upon the total

Fig. 1. Dog 3. October 19, 1920. Tracing from cranial plethysmograph. Upper line showing effect of 40°C. bath; lower line, effect of removal from bath after 22 minutes.

Fig. 2. Same experiment as figure 1. Upper line showing effect of cold bath (8°C.); lower line, effect of removal from same after about 25 minutes.

solids of the blood. These are illustrated in figures 3 and 4 where one sees constantly a decided increase in the water content of the blood with a bath the initial temperature of which was 40°C., and a corresponding decrease with a cold bath. Owing to the cooling effect of the evaporation from the skin, removal of the dog from the hot bath in every case gave decrease in the water content of the blood.

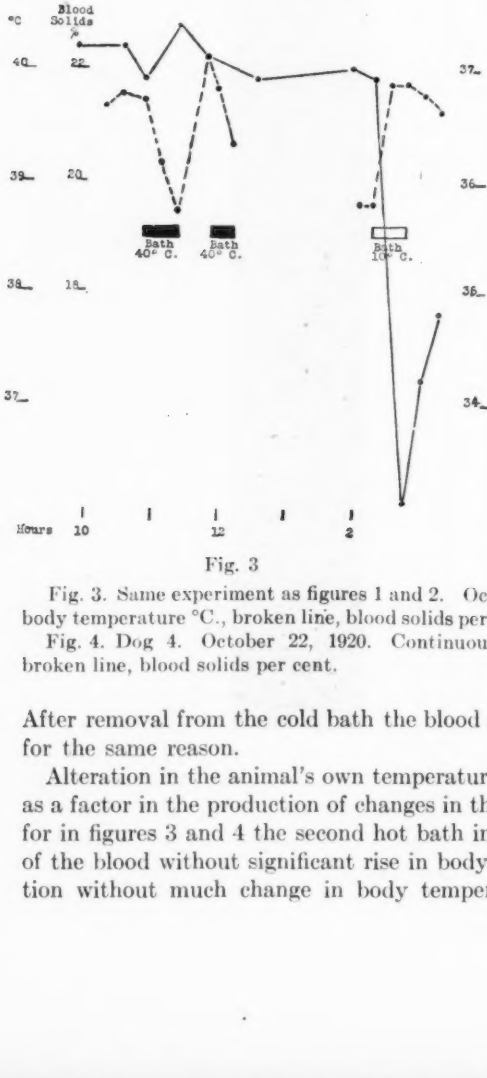


Fig. 3

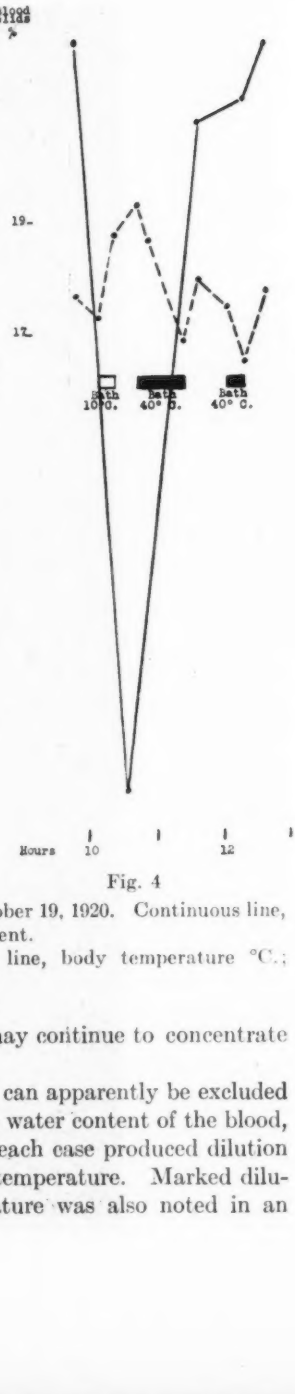


Fig. 4

Fig. 3. Same experiment as figures 1 and 2. October 19, 1920. Continuous line, body temperature °C., broken line, blood solids per cent.

Fig. 4. Dog 4. October 22, 1920. Continuous line, body temperature °C.; broken line, blood solids per cent.

After removal from the cold bath the blood may continue to concentrate for the same reason.

Alteration in the animal's own temperature can apparently be excluded as a factor in the production of changes in the water content of the blood, for in figures 3 and 4 the second hot bath in each case produced dilution of the blood without significant rise in body temperature. Marked dilution without much change in body temperature was also noted in an

experiment on dog 6, which had been trephined on the previous day. The animal was exposed to a bath having an initial temperature of 40°C. for a period of one and a half hours. The most marked increase in the water content of the blood took place during the last half hour. The animal had been restless during the first part of the immersion. While the blood solids fell from 19.4 to 17.1 per cent the rise in body temperature was only 0.3°C. in the ninety minutes. As regards the influence of cold, experiments conducted later (12) have shown that, where a bath at 20°C. is substituted for the 10°C. bath, marked concentration of the blood is also obtainable, and without reduction in body temperature.

Relation of water content of blood to brain volume: Comparing the effects of the baths upon the brain volume with those upon the blood solids, a certain parallelism cannot be denied. It seems possible that under the influence of the cold stimulus water leaves the blood for the brain, heat again tending to draw it back from the brain to the blood. This view does now however withstand criticism at all points. Particularly instructive are the effects of the first hot bath given to dog 4. The tremendous increase in brain volume at first must be associated with the animal's temperature having been very low at the time of immersion; yet while this increase is still marked the blood had already begun to dilute at the usual rate as shown by figure 4. Again, we do not precisely know all of the factors concerned in these plethysmographic changes; blood pressure in particular is doubtless involved at times.

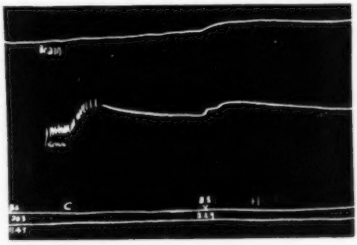


Fig. 5. Effects of cold and heat under morphine-chloretone anesthesia. Upper line, cranial plethysmograph. In center, blood pressure tracing. *C*, cold applied. *H*, heat applied. *B.S.*, blood sample taken (20.3 per cent at 11:45 a.m. 22.3 per cent at 12:05 p.m.)

That brain volume may, however, increase under the influence of the cold stimulus independently of blood pressure changes is shown by further experiments under morphine-chloretone anesthesia. As a result of cooling an anesthetised dog by sprinkling the chest and abdomen with ice water while a strong current of air is being thrown across the animal, one sees in figure 5 (dog 8), that the usual increase in brain volume was produced. This was accompanied during the first four or five minutes by a considerable blood pressure rise, but the increase in brain volume continued from this time on, whilst the blood pressure gradually fell. As a result of twenty minutes exposure to cold in this fashion, the blood solids increased from 20.3 to 22.3 per cent; the usual effects of cold upon brain volume and blood concentration were therefore seen.

With heating, on the other hand, however, typical effects could not be obtained in this anesthetised dog. A warm stove was placed beneath the animal and an electric pad above, and the whole well covered, but the fall in brain volume, as will be seen from the latter part of figure 5 was insignificant although the temperature of the air about the dog's skin became as high as 50°C. The animal was heated in this way for eighty minutes at the end of which time the blood solids were practically unchanged,—22.4 per cent.

Further experiments under anesthesia: The last part of the experiment just cited indicates that anesthesia may inhibit the function of the water shifting mechanism. To test this point further two similar experiments were performed under morphine-chloretone anesthesia. The same methods of cooling and heating as just described were applied.

The first animal gave typical but not extensive responses to the first applications of cold and heat which were continued for fifty and ninety minutes respectively. A later application of cold produced a still less marked concentration of the blood, and this was followed by a practically ineffective application of heat. In this experiment the brain showed little if any change in volume as indicated by the plethysmographic tracing, when the heat was applied. On the other hand a response to cold was seen both times. (Lack of correlation between brain volume and blood pressure was also seen.) It appears possible therefore to obtain some response to cold both as regards blood concentration and brain volume under morphine-chloretone anesthesia.

A further change which we have usually seen as the result of a cold stimulus consists in a very marked diminution of the carotid pulse amplitude (fig. 5), explainable on either or both of the assumptions of vasoconstriction or diminished strength of cardiac impulse.

With deep anesthesia there is no question that heat and cold are both ineffective stimuli for changes in brain volume and blood solids. The latter point was well illustrated in dog 10, where neither heat nor cold produced a change in blood solids of over 0.2 per cent. At two different times in this experiment control estimations of the blood solids were made from the samples taken from the carotid artery. In each case the determination of 23.5 per cent in the carotid artery corresponded to 23.8 per cent in blood taken practically simultaneously from the ear vein in the usual manner.

CONCLUSIONS

1. Normally the blood solids of fasting dogs at rest in a stable environment are subject only to insignificant variations. Over six hours changes greater than 1 per cent should not be seen, nor should the hour to hour variation exceed 0.5 per cent.

2. Previous observations that the stimuli of cold and moderate heat give rise respectively to increase and decrease in the red cell count, hemoglobin content, viscosity, etc., of the blood, have been confirmed by the method of determining the total blood solids in dogs. The results may certainly be interpreted as indicating the relative amount of water in the actively circulating parts of the blood stream. The importance of this as bearing upon the peripheral blood flow, and upon the amount of water available for evaporation from the body is self-evident. The relation of the blood solids to the mechanism of heat-regulation suggested by our previous studies of fever, antipyretic drugs, etc., is thus confirmed.

3. Effects upon normal dogs of more extreme heat stimuli than are afforded by immersion at about 40°C. in a saline bath of small volume, allowed to cool gradually, are not discussed in this paper.

4. The response of the brain volume to cold and hot baths is usually an increase in the former, and a decrease in the latter. This may account to some extent for the fate and source of water passing respectively out of, and into the blood. The brain volume changes are not necessarily associated with alterations in blood pressure.

5. Morphine-chloretone anesthesia not only tends to make dogs poikilothermic, but interferes simultaneously with their water shifting mechanism.

BIBLIOGRAPHY

(1) Barbour and Baretz: Proc. Soc. Exper Biol. and Med., 1920, xvii, 209.
(2) Barbour and Freedman: This Journal, 1921, lvii, 387.
(3) Barbour and Herrmann: Journ. Pharm. Exper. Therap., 1921, xviii, 165.
(4) Barbour and Howard: Proc. Soc. Exper. Biol. and Med., 1920, xvii, 148, 150.
(5) Matthes: Lehrbuch d. klin. Hydrotherapie. Jena, Fischer, 1903, 469.
(6) Bazett and Haldane: Proc. Physiol. Soc., 1921, Journ. Physiol., lv.
Young, Breinl, Harris and Osborne: Proc. Roy. Soc., London, 1920, xciB, 111.
(7) Müller and Veiel: Sammlung klinischer Vorträge, Leipzig, 1910–11, 606, 630.
(8) Strassburger: Med. Klinik, 1913, ix, 752.
(9) Hill: Journ. Physiol., 1897–98, xxii, (Proc.) xxvi–xxix.
(10) Keith, Rowntree and Geraghty: Arch. Int. Med., 1915, xvi, 547.
(11) Lamson and Nagayama: Journ. Pharm. Exper. Therap., 1920, xv, 331.
Lamson and Rosenthal: This Journal, 1923, lxiii, 358.
(12) Barbour and Tolstoi: This Journal, 1923, lxvii, 378.
(13) Gross and Kestner: Zeitschr. f. Biol., 1919, lxx, 187.
Cohn: Zeitschr. Biol., 1919, lxx, 366.
Eckert: Zeitschr. Biol., 1920, lxxi, 137.

HEAT REGULATION AND WATER EXCHANGE

II. The Rôle of the Water Content of the Blood, and its Control by the Central Nervous System[1]

HENRY G. BARBOUR and EDWARD TOLSTOI

From the Department of Pharmacology, McGill University, and the Department of Pharmacology, Yale University

Received for publication September 24, 1923

In the preceding paper one of us (1) demonstrated that normal dogs respond to cold (10°C.) and hot (40°C.) baths respectively, by the loss and gain of water in the blood.

The chief object of the investigations to be here reported was to determine whether or not the transfer of water to and from the blood under the influence of thermal stimuli is dependent upon the maintenance intact of the heat regulating centers of the central nervous system. In other words, will animals made poikilothermic by operation on the central nervous system still respond to thermal stimuli by the same changes in the blood as are seen in normal animals? A negative answer to this question will be anticipated when it is recalled from the previous paper that a number of experiments upon animals anesthetized with morphine and chloretone showed apparent impairment of the regulation of the water content of the blood.

As the work progressed it was found necessary to define more clearly in normal dogs the relations between bath temperature and blood solids. It was especially necessary to observe their behavior in baths kept at an exactly even temperature throughout the period of immersion.

Methods. All the dogs used in this work were of about 10 kilos weight, and of medium length hair. They were kept under excellent hygienic conditions, being allowed to run out of doors on most pleasant days, and being fed on a constant adequate diet which consisted of an evening meal each day of canned meat, 8 ounces, bread, 4 ounces, lard, 2 ounces. Water was always available except during actual periods of experimentation when it was always withheld. No dog was ever experimented upon within sixteen hours after feeding.

Thermal stimuli: The first few experiments were performed with the same type of bath described in the first paper, except that tap water was

[1] The expenses of this research were defrayed from a grant from the Elizabeth Thompson Science Fund.

used instead of salt solution. The animal sat upright in a galvanized metal pail just large enough to accommodate it and was kept wholly immersed up to the neck. An attendant was always present throughout, and after the effect of the initial plunge had subsided, disturbance of any sort was extremely rare.

After a few preliminary experiments it was deemed advisable to resort to baths of a more constant temperature, which was done by employing a much larger body of water and by regulation with a burner or by additions of cold water as required.

The dog and the entire water pail were placed inside of a second larger metal bath holding approximately 400 liters of water, and measuring approximately $5 \times 2 \times 1\frac{1}{2}$ feet. The water in both vessels was heated to the desired degree several hours before experimentation, and by means of careful attention and hand stirring it was found possible to keep it regulated within 0.2 or 0.3°C. of the required temperature for as long a period as desired. From this time on, only one temperature was employed in a given experiment. The room temperature in each experiment was kept at approximately 22°C.

Observations: Besides the general behavior of the animal, and the presence or severity of panting, attention was concentrated upon following closely the dog's rectal temperature and blood solids. The temperature was recorded every five minutes by means of a small galvanometer (Leeds and Northrup, no. 2400A) in series with two constantan-copper thermo-couples. The first of these was kept in distilled water in a well insulated Dewar vacuum flask; the other junction was kept at a distance of 8 cm. within the rectum of the dog, the wires here being protected by rubber tubing, and held firmly in position by bandages or cords passed around the body of the animal. A highly accurate thermometer placed in the vacuum flask was always read simultaneously with the galvanometer reading. The water in the Dewar flask was kept at about 35°C., under which condition the galvanometer calibration was several times checked. As an additional precaution the dog's temperature was always taken with a mercury thermometer just before, and just after, each immersion.

Operations: The operations on the nervous system were performed with the most stringent aseptic technique. Two varieties of operations were carried out, the one described as decerebration consisting of trephining the subtemporal region of the skull, enlarging the opening, removal of both cerebral lobes by ablation with the blunt end of a scalpel. What remained of the basal ganglia was then removed to the level of the corpora quadrigemina except for a thin layer of tissue at the base of the optic thalami. The usual precautions against hemorrhage were taken. The best results with these animals were obtained by exposing them to baths within a few hours of operation.

Nearly all of the operations were of the "cord" variety. The arch of the 6th cervical vertebra was first removed, the dura opened, and the spinal cord transected at this level. The usual procedure was to remove completely a length of cord amounting to about 1 cm., but in the first few experiments transection alone was done.

The "cord" dogs were kept alive for as many days as needed by simply wrapping in blankets and keeping near a small gas stove. The diet of these animals was changed after operation to 500 cc. of milk each day, given by stomach tube when necessary.

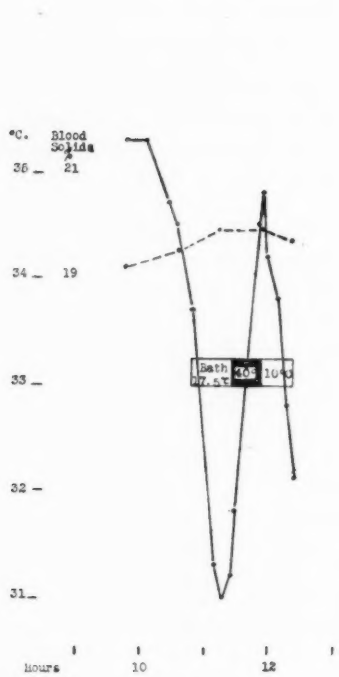


Fig. 1

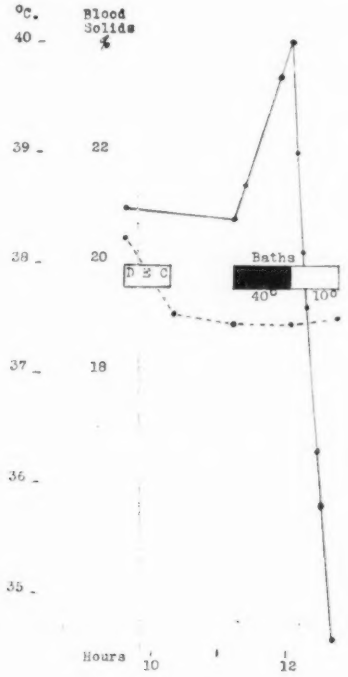


Fig. 2

Fig. 1. Dog. 21. Effects of baths at various temperatures upon dog made poikilothermic by "cord" operation. Continuous line, body temperature; broken line, blood solids.

Fig. 2. Dog 34. Effects of baths at various temperatures upon dogs made poikilothermic by "decerebration" operation. Continuous line, body temperature; broken line, blood solids. Time of operation indicated by *DEC*.

RESULTS. It was not difficult to establish the probability of the general proposition that dogs made poikilothermic by the above described operations

fail to respond normally to thermal stimuli by active shifting of water into or out of the blood. This is illustrated in figures 1 and 2, representing respectively the effects of baths upon a "cord" dog and a "brain" dog. It will be recalled that normal dogs exposed to baths having initial temperatures of 40°C. and 10°C. respond respectively by a fall and a rise in the blood solids amounting to 1 per cent or more of the total blood solids within thirty minutes. In these two experiments the greatest change noted at these temperatures was a fall in blood solids of 0.2 per cent following exposure to 10°C. bath for thirty minutes. The same dog had shown a rise of 0.4 per cent in a bath of 17.5°C. The variations in body temperature were extreme, as would be expected from exposure of poikilothermic dogs to these temperatures.

Effects of cold baths at constant temperatures. The effects of cold baths at constant temperatures maintained from thirty to fifty-five minutes, are summarized in table 1. These experiments all gave clear-cut results.

TABLE 1

Effects of cold baths on blood concentration

DOG NUMBER	OPERATION	BATH TEMPERATURE	BLOOD SOLIDS		RECTAL TEMPERATURE		
			Before	After 40 minutes	Before	After 40 minutes	After 55 minutes
		°C.	*per cent*	*per cent*	°C.	°C.	°C.
48	Control	20	19.3	20.3	39.9	40.9	
23	Control	20	20.4	22.6	38.3	38.7	
27	Control	20	14.7	16.5	39.4	37.6	38.7
42	Cord	20	18.1	18.9	36.5	32.6	
27	Cord	20	17.3	17.6	37.5	34.6	
100	Cord	20	17.9	17.4	39.2	32.6	
21	Cord	17.5	19.5	19.9	34.5	31.0	
21	Cord	10	19.9	19.7	34.8	32.1	
34	Brain	10	18.9	19.0	40.0	34.6	

The fact was readily established that 20°C. baths of such duration do not permanently lower the temperature of normal dogs; in fact, in two out of three animals the rectal temperature was distinctly increased in forty minutes, while in the third control occurred a rapid partial recovery from an initial downfall of temperature.

An increase in the blood solids similar to that seen in the 10°C. baths in the previous paper was seen in each experiment. The minimal increase was 1 per cent of the total solids; the maximal increase was 2.2 per cent.

In the same table are presented the results of six experiments upon poikilothermic dogs, all exhibiting a rapid diminution in body temperature. The blood solids however showed no significant increase in any case except the first, dog 42, which did show an increase of 0.6 per cent,—less than the minimum normal increase. It is clear from these results that coincident

with their failure to regulate against a cold environment, poikilothermic dogs are also unable to concentrate the blood in response to cold. The result in dog 34 (decerebrated) is illustrated in figure 2, already referred to, while figure 3 illustrates the responses of a normal dog (no. 23) to the constant 20°C. bath.

Effects of constant high temperature baths in normal dogs. (Tables 2, 3, and 4.) In looking over the control warm bath experiments it will be seen that a 38°C. bath given under the conditions we have described, did not usually alter significantly the body temperature of the dog, while baths of the two higher temperatures each caused an increase in the body temperature which was usually less than 1° in the 40° bath, and usually 1° or over in the 42° bath.

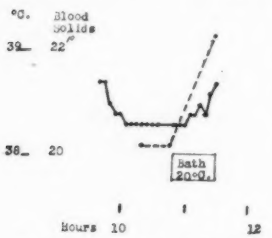


Fig. 3. Dog. 23. Effects of constant temperature bath at 20°C. upon normal dog.

The 38°C. bath produced no change in the blood solids of normal dogs, thus indicating that this temperature is resisted without calling into play the water shifting mechanism. This is not surprising when we consider that it is a little below the dog's own temperature, and that the projecting head and neck were exposed to the cooling influence of the room atmosphere. Continuous panting was not noted in dogs at this temperature.

TABLE 2

Effects of 38°C. baths on blood concentration

DOG NUMBER	OPERATION	BATH DURATION	BLOOD SOLIDS			RECTAL TEMPERATURE		
			Before	After	Difference	Before	After	Difference
		min.	*per cent*	*per cent*	*per cent*	*°C.*	*°C.*	*°C.*
42	Control	50	21.1	21.1	0.0	38.8	39.4	+0.6
48	Control	40	19.0	19.0	0.0	39.0	39.0	0.0
54	Control	40	19.5	19.8	+0.3	38.3	37.8	−0.5
58	Control	60	19.8	20.0	+0.2	38.9	39.1	+0.2
		90		21.0	+1.2		38.9	0.0
27	Cord	40	17.7	17.4	−0.3	38.0	38.7	+0.7
54	Cord	60	19.2	17.9	−1.3	29.0	36.7	+7.7

The 40°C. bath produced in the majority of cases a definite dilution of the blood. This result, however, was not seen constantly, as in the case of the small bath allowed to cool down, described in the previous paper. In four experiments, dogs 71, 78, 100, 83, it will be seen that at one time or other a decided increase even occurred. This increase in the blood solids was usually associated, however, with a considerable rise in the body temperature, showing that the facilities for cooling (factors of humidity and air motion) were not as good as usual. Following individual experiments by

half-hours, it will be seen that the best instances of dilution occurred simultaneously with the best success of the dog in keeping his temperature at a constant level, showing again the rôle of blood dilution in regulating against a warm environment. The majority of these dogs exhibited panting most of the time, but the severity of this does not show any particular parallelism to the other results.

TABLE 3

Effects of 40°C. baths on blood concentration

DOG NUMBER	OPERATION	BATH DURATION	BLOOD SOLIDS			RECTAL TEMPERATURE		
			Before	After	Difference	Before	After	Difference
		min.	*per cent*	*per cent*	*per cent*	*°C.*	*°C.*	*°C.*
48	Control		17.5	17.2	−0.3	38.6	38.9	+0.3
54	Control		21.4	17.1	−4.3	38.8	39.0	+0.2
54	Control	40	20.1	19.8	−0.3	38.0	39.4	+1.4
58	Control	60	17.2	15.9	−1.3	38.6	39.3	+0.7
58	Control		17.8	17.5	−0.3	38.3	39.3	+1.0
61	Control	60	16.2	15.8	−0.4	39.6	40.0	+0.4
71	Control	30	22.0	19.8	−2.2	38.2	39.3	+1.1
		60		20.5	−1.5		39.6	+1.4
		90		23.6	+1.6		39.8	+1.6
78	Control	30	19.5	18.7	−0.8	38.6	39.3	+0.7
		60		21.3	+1.8		39.3	+0.7
		90		18.2	−1.3		39.2	+0.6
100	Control	60	18.9	19.4	+0.5	38.9	39.2	+0.3
		90		18.4	−0.5		39.7	+0.8
83	Control	40	21.0	22.1	+1.1	38.4	40.6	+2.2
21	Cord	30	19.9	19.9	0.0	31.0	34.8	+3.8
71	Cord	60	20.0	20.4	+0.4	34.8	38.1	+3.3
		120		19.2	−0.8		40.1	+5.3
48	Cord		17.5	17.2	−0.3	36.0	37.4	+1.4
100	Cord	60	17.4	17.2	−0.2	33.6	38.1	+4.5
		90		17.1	−0.3		39.4	+5.8
100	Cord	70	16.2	16.1	−0.1	38.9	41.2	+2.3
34	Brain	50	18.9	18.9	0.0	38.5	40.0	+1.5
4	Brain	40	18.0	16.9	−1.1	35.9	38.9	+3.0

The highest temperature (42°C.) baths resulted on the whole in concentrating the blood as frequently as diluting it. At this temperature continuous panting was constantly seen, the body temperature was usually more strikingly increased, in short, the dog had as a rule more limited opportunity for giving off heat than a 40°C. dog.

In 42° as well as in 40° baths the dogs usually exhibited an increase in the blood solids when the temperature rose rapidly but dilution of the blood seemed to hold the body temperature at a constant level. These facts are well shown in figures 4 and 5.

TABLE 4

Effects of 42°C. baths on blood concentration

DOG NUMBER	OPERATION	BATH DURATION	BLOOD SOLIDS			RECTAL TEMPERATURE		
			Before	After	Difference	Before	After	Difference
		min.	*per cent*	*per cent*	*per cent*	*°C.*	*°C.*	*°C*
54	Control	30	18.4	20.2	+1.8	38.2	40.6	+2.4
		60		19.1	+0.7		40.9	+2.7
		90		20.1	+1.7		41.0	+2.8
58	Control	30	19.9	19.4	−0.5	39.3	39.7	+0.4
		60		19.4	−0.5		39.8	+0.5
61	Control	60	17.0	17.4	+0.4	39.6	40.1	+0.5
		90		15.8	−1.2		40.0	+0.4
70	Control	90	20.8	21.6	+0.8	38.5	40.1	+1.6
78	Control	60	18.2	18.2	0.0	38.4	39.5	+1.1
		90		17.1	−1.1		39.4	+1.0
83	Control	30	20.9	23.1	+2.2	38.4	39.4	+1.0
		90		23.4	+2.5		39.4	+1.0
100	Cord	30	16.1	15.8	−0.3	37.9	39.0	+1.1
		60		15.0	−1.1		41.5	+3.6
		90		15.8	−0.3		41.5	+3.6

Later experience has convinced us that lack of uniformity in the results with high temperature baths is to be attributed to differences in relative humidity and in air movement. Doctor Lozinsky (2), in the paper which

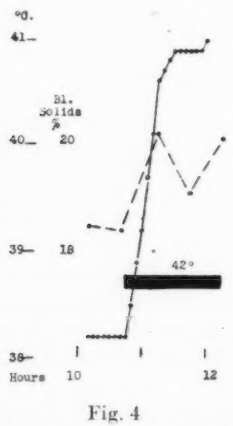


Fig. 4

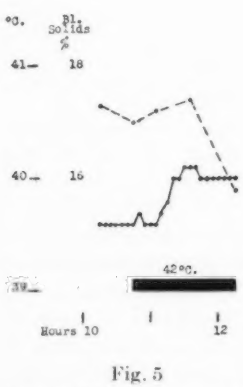


Fig. 5

Fig. 4. Dog 54. Effects of constant temperature at 42°C. bath upon normal dog.
Fig. 5. Dog. 61. Effects of constant temperature at 42°C. bath upon normal dog.

follows this, describes work on dogs in warm atmospheres. He divided his experiments into two groups according as the environment was moist or dry. In each group he found that at the highest environmental temperatures which dogs can tolerate without losing control of body temperature there is always a definite dilution of the blood, while just above the point where the regulation breaks and the body temperature becomes increased, concentration of the blood and marked panting result. Naturally these levels of (*a*) *dilution of the blood with maintained temperature*, and (*b*) *concentration of the blood with augmented temperature*, fall at different room temperature levels according as the atmosphere is dry or moist.

Effects of constant high temperature baths in poikilothermic dogs. Turning now to the operated animals (tables 2, 3, 4,) one sees at all three bath temperatures the inability of the animal to maintain a constant body temperature as shown by rapid increases in the same.

The blood solids on the other hand in six out of ten poikilothermic dogs exhibited at these bath temperatures a striking tendency to remain constant. This is in full accord with the above demonstrated failure of the water shifting mechanism on exposure of such dogs to cold.

This constancy in the level of the blood solids in poikilothermic dogs exposed to high temperatures is broken in four experiments, namely: in table 2, dog 54 (cord); table 3, dog 71 (cord), second hour, and dog 4 (brain); and table 4, dog 100, second half-hour. It is interesting to note that this unexpected dilution of the blood always accompanies an extremely rapid rise in body temperature. The three dogs of the four which showed the greatest blood dilution exhibited an increase in body temperature of over 4°C. per hour (fig. 6). These four exceptions may therefore exemplify the effects of body temperature upon the water content of the blood, rather than the effects of water shifting upon body temperature. This con-

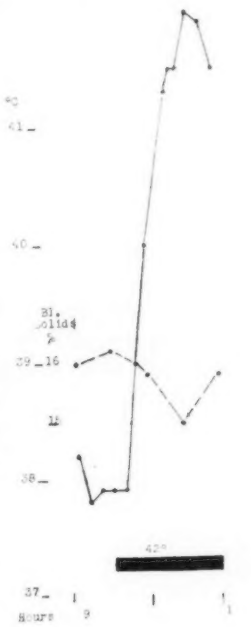


Fig. 6. Dog 100. Effects of constant temperature 42°C. bath upon dog made poikilothermic by "cord" operation.

Note comparative constancy of blood solids except for one reading at peak of body temperature. Dilution may have been due to extremely rapid rise to an abnormally high temperature. This dog panted from 10 to 11 a.m. owing probably to the effect of the heated blood upon temperature and respiratory centers which, with their efferent connections, were intact.

jecture received support from the fact that the first mentioned of the above cases occurred in a 38°C. bath, in which condition one never gets any change in the concentration of the blood of a normal dog (table 2). The exceptional cases of blood dilution in poikilothermic dogs are not therefore due to persistence of the water shifting mechanism.

SUMMARY OF RESULTS WITH CONSTANT TEMPERATURE BATHS. 1. Baths at 20°C. were tolerated without significant changes in rectal temperature. The blood solids were increased by from 1 to 2 per cent.

2. Baths at 38°C. were tolerated by normal dogs without significant changes in either rectal temperature or blood solids.

3. Baths at 40°C. and 42°C. tended to raise the body temperature of all dogs exposed one to one and a half hours. The average increase was a little less than 1°C. in the former case, and a little over 1°C. in the latter case. During the periods of best regulation the blood usually became more dilute, on the other hand rapid increases in body temperature were associated with concentration of the blood.

4. Where increased heat loss is possible dogs appear to regulate by blood dilution. Where it is impossible the blood concentrates, not necessarily as the passive result of panting only, but also possibly as an attempt to insulate the body against extreme heat.

5. By the brain or cord operations described, dogs were not only made poikilothermic, but were also deprived of the water shifting mechanism.

Exposed to 20°C. constant temperature baths such dogs exhibited a rapid decline in body temperature without significant alteration in the blood solids. Exposed to 38°, 40° and 42°C. constant temperature baths poikilothermic dogs exhibited a rapid rise in body temperature. In six cases out of ten no significant changes in the blood solids occurred. In the other four cases the blood dilution observed accompanied a particularly rapid temperature rise, which is regarded in these cases as its cause.

SUMMARY OF ARGUMENT FOR THE IMPORTANCE OF THE WATER CONTENT OF THE BLOOD IN HEAT REGULATION

In conclusion it seems appropriate to summarize the present evidence in support of the theory that the water content of the blood is an essential factor in the maintainance of a constant body temperature (3).

1. Experiments with pyretic agents, especially Bacillus coli vaccine (4) and cocaine (5), have shown that the onset of fever (which involves abnormal stimulation of the regulation against cold) is accompanied by concentration of the blood.

2. Experiments with antipyretic agents (6), (7) have shown that the occurrence of antipyretic action is paralleled by blood dilution, and failure of antipyresis is accompanied by absence of change in the blood solids.

3. Evidence from the present paper shows that concentration of the blood upon exposure to cold occurs while the animal's temperature remains normal, or even rises, and therefore cannot be due to cooling of the actively circulating blood. Similarly dilution of the blood in response to hot baths is most frequently seen precisely when the normal animal's temperature is most constant.

4. The blood solids on the other hand remain most constant in "cord" and "brain" dogs, animals whose blood temperatures fluctuate the most violently of any. It seems difficult to avoid the conclusion that loss of heat regulation by operation is intimately connected with impairment of the water shifting mechanism.

5. While not to be regarded as direct evidence, it is corroborative to find the logic of the situation satisfied from a teleological point of view. For blood concentration by hampering both peripheral blood flow and water loss will protect the body either from losing or acquiring too much heat at decidedly cold and hot temperatures respectively. Furthermore the "moderately warm" environmental temperature range which approximates the temperature of the animal body and demands the greatest facilities for radiation presents precisely the condition where dilution of the blood is found.

The rôle of atmospheric humidity in this connection has been investigated by Doctor Lozinsky, whose report follows.

BIBLIOGRAPHY

(1) Barbour: This Journal, 1923, lxvii, 366.
(2) Lozinsky: This Journal, 1923, lxvii, 388.
(3) Barbour: Physiol. Reviews, 1921, i, 295.
(4) Barbour and Howard: Proc. Soc. Exper. Biol. and Med., 1920, xvii, 148.
(5) Barbour and Moise: Journ. Pharm. Exper. Therap., 1922, xix, 270.
(6) Barbour and Herrmann: Journ. Pharm. Exper. Therap., 1921, xviii, 165.
(7) Barbour and Howard: Proc. Soc. Exper. Biol. and Med., 1920, xvii, 150.

HEAT REGULATION AND WATER EXCHANGE

III. The Effects of "Dry" and "Moist" Heat upon the Body Temperature and Blood Concentration of Dogs[1]

EZRA LOZINSKY

From the Department of Pharmacology, McGill University

Received for publication September 24, 1923

The experiments to be described below were undertaken to study the respective effects of high and low relative humidity upon the changes which a hot atmospheric environment produces in the body temperature and water content of the blood. Barbour and Tolstoi (1) have shown that dogs exposed to cold baths concentrate, and when exposed to hot baths dilute, their blood. They found that the best bath temperature for obtaining dilution of the blood was about 40°C., at 38°C. no change took place, while at 42°C. concentration was more frequently observed. They suggested that this concentration of the blood may be in effect a regulation against overheating in the same manner as against cooling, the animal reducing the amount of fluid in the peripheral circulation and hence in contact with the unfavorable environmental temperature.

Under tropical conditions with high temperature and humidity, Young, Breinl, Harris and Osborne (2) found the blood sometimes diluted and sometimes concentrated; when however their subjects were confined in a hot chamber with very high humidity concentration of the blood uniformly occurred.

Where the environmental temperature is high Berti, cited in (3), observed vasoconstriction and increased viscosity of the blood. Hunt (4) showed that several liters may be lost by sweating without appreciably diminishing the water content of the blood.

The relative humidity of the atmosphere seems to be largely responsible for the variability in the results cited above. It was hoped in the present experiments, by substituting for the baths used by Barbour and Tolstoi, the atmosphere of a constant temperature chamber of which the relative humidity could be controlled, to establish more definitely the conditions under which blood dilution and blood concentration take place in a warm environment; and if possible to indicate the factors involved in producing the variations.

[1] From a thesis presented in candidacy for the degree of M.Sc., McGill University, 1923.

METHODS. The hot chamber used in these experiments is a slight modification of that described in catalogue 16 of the Chicago Surgical and Electrical Co. Our incubator includes one additional stove, arrangement of the ventilating fan to run continuously or intermittently as desired, and a one-half centimeter brass tube communicating with the outside, through which steam can be blown when it is desired to raise the humidity of the chamber. The inside dimensions are: length, 1.83 meters; width, 1.2 meters; height, 1.96 meters, giving a capacity of 4.3 cubic meters. During the time that no active ventilation is in progress, slow interchange of the air in the cabinet with that on the outside is still possible through the ventilating shafts. This, together with the size of the room indicates that during an experimental period of a few hours the accumulation of CO_2 is not a factor to be considered.

The cabinet contains wet and dry-bulb thermometers, with a small electric fan behind them, and a wire animal cage. The small fan behind the thermometers was kept running continuously during each experiment, except in a few instances where the heat thus generated tended to raise the temperature of the room above that desired. On these occasions it was run for about a minute previous to taking the wet-bulb reading. The animal was shielded from the direct air current by a sheet of cardboard. Thus temperature, relative humidity and air motion were all controlled.

The animals used were all short-haired dogs in good condition weighing between 9 and 11 kilograms, they were kept at room temperatures varying between 22° and 25°C., fasted (but allowed water) for twenty-four hours before the experiment; during the experiment water was also withdrawn.

The animal was weighed and at hourly, or more frequently half-hourly intervals, duplicate samples of blood were drawn and rectal temperature recorded; the animal was then weighed again and transferred to the hot room where it was kept for periods varying from one to two hours, at half-hour intervals duplicate samples of blood were withdrawn and rectal temperature recorded; after removal of the animal from the hot room it was again weighed and at half-hourly intervals duplicate samples of blood were again taken and rectal temperature recorded; at the end of the hour the dog was weighed again.

For determination of changes in water content the blood solids method was used. The blood was drawn from the saphenous vein just above the ankle through a hypodermic needle into a weighed watch glass, immediately covered and weighed, dried at 110°C., then weighed again and the percentage of blood solids calculated. Duplicate samples were found to check with a high degree of accuracy.

EXPERIMENTAL DATA. To clarify the description of the results, it may be well at the outset to enumerate the four levels of the reaction to warming which were distinguished when the results of the various experiments were

analyzed. They are given in order of increase of environmental temperature, other conditions being constant: *a*, level of no change; *b*, level of blood dilution with no rise in body temperature; *c*, transition level, no change in blood solids and no increase in body temperature, but marked panting; *d*, level of blood concentration, with marked rise in body temperature.

The experimental data my be conveniently divided into two parts:

1. Effects of various room temperatures with high relative humidity, 80 to 94 per cent.

2. Effects of various room temperatures with low relative humidity, 21 to 51 per cent.

HIGH RELATIVE HUMIDITY. In the experiments involving high relative humidity the constant room temperature employed ranged from 27.8° to 41°C.

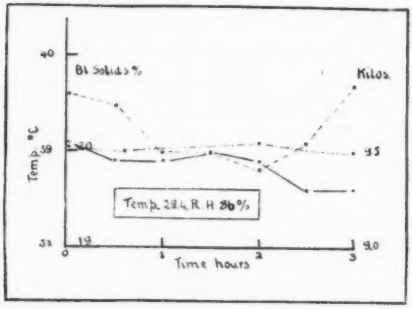


Fig. 1. Continuous line, body temperature; broken line, percentage blood solids; dots and dashes, body weight.

Level of no change. No experiments were done at this level which is assumed to include ordinary room temperatures 20° to 25°C.

Dilution level. Between 27.8° and 30°C. the animal rested quietly in the cage, there was no panting, essentially no change in body temperature, the blood solids on the other hand showed a decrease ranging from 1.1 per cent at 27.8°C. to 0.2 per cent at 30°C., there was no loss in body weight.

Below is a protocol, typical in form of all the others, recording the observations of one such experiment (no. 30). These observations are graphically illustrated in figure 1.

Transition level. Between 30° and 33.1°C. there was a moderate degree of panting, practically no change in body temperature, and but a slight change in blood solids, a decrease of from 0.1 to 0.2 per cent; no significant change in body weight was noted.

On removal of the animal from the hot room after both conditions above described, the blood solids very often showed some degree of concentration. In explanation it might be said that during confinement in the hot room the animal found itself in almost ideal conditions for a state of rest, i.e., an environment just a little below body temperature, and humid. Consequently on return to ordinary room temperature the reaction is as to cold—the blood concentrates (see experiments 32, 30 and 31, table 1).

Experiment 30. April 19, 1923.

Dog 80. Description: Male white mongrel, short-haired.

Time: 2:00 p.m. *Weight:* 9.530 kgm.

TIME	ROOM TEMPERATURE		AVERAGE RELATIVE HUMIDITY	DOG'S TEMPERATURE	BLOOD SOLIDS
	Dry bulb	Wet bulb			
	°C.	°C.	*per cent*	°C.	*per cent*
2.15	22.0			39.1	20.5
					20.7
3.00				38.9	20.6
					20.3
3.10	27.3	25.1	Dog placed in hot room		Weight 9.520 kgm.
3.40	29.1	27.8		38.9	19.9
			86		20.0
4.10	29.5	27.2		39.0	20.0
					20.1
4.40	29.4	27.5		38.9	19.7
					19.8
			Dog taken out of hot room.		Weight 9.550 kgm.
5.10				38.6	20.0
					20.2
5.55	22.0			38.6	20.7
					20.7
				Weight 9.500 kgm.	

Notes: No panting throughout experiment. No ventilation.

Level of concentration. When the temperature of the room was set above 33°C., the animal panted very vigorously, the body temperature increased as though the animal were practically poikilothermic, in other words, every degree of increase in room temperature elevated the animal's temperature nearly one degree. Here the blood solids showed considerable increase, ranging from 2.3 to 3.6 per cent. The animals lost 100 to 300 grams in weight, apparently by giving off water through the respiratory tract and evaporation from the mucous membrane of the mouth in spite of the air being as saturated as possible.

The body temperature may rise rapidly to over 43.5°C. and the animal be on the verge of collapse, and if exposed longer than one hour to one hour and a half, death may occur, as in experiment 19. The animal in this case was exposed to saturated air at 41°C. for one hour, and succumbed one half-hour after removal from the hot chamber. Ordinarily, however, on removal from the hot chamber the animal's temperature rapidly fell to normal and coincidentally the blood solids also tended to reach their normal level.

Low relative humidity. In the experiments involving low relative humidity the constant room temperatures employed ranged from 33.5°C.

to 54.5°C. It may be stated at the outset that all reaction levels under the conditions of low relative humidity lie at a higher temperature than when the relative humidity is high.

Level of no change. As far as our observations go, this level extends between room temperature of 22° and 35.5°C., the upper limit. Thus in two experiments, one at 33.5°C., and the other at 35.5°C., there was a very moderate degree of panting, body temperature was not significantly

TABLE 1

Effects of various room temperatures with high humidity (80 to 94 per cent) on body temperature and blood solids of dogs

	EXPERIMENT NUMBER						
	32.*	30.*	31‡*	29‡	18†	25†	19$†
Temperature °C. of hot room	27.8	29.4	30.2	33.1	37.0	40.1	41.0
Normal temperature °C. of animal	38.4	38.9	38.4	38.3	38.4	38.4	39.0
Temperature °C. of animal after one hour's exposure in hot room	38.7	39.0	38.9	38.5	41.3	42.8	43.3
Net change in animal's temperature after one hour's exposure in hot room	+0.3	+0.1	+0.5	+0.2	+2.9	+4.4	+4.7
Temperature °C. of animal one hour after return to ordinary room temperature	38.4	38.6	38.6	38.3	38.5	38.7	
Normal blood solids per cent	20.0	20.5	19.4	20.3	21.2	18.6	22.5
Blood solids per cent after one hour's exposure in hot room	18.9	20.0	19.2	20.2	23.8	22.4	24.8
Net change in blood solids per cent after one hour's exposure in hot room	−1.1	−0.5	−0.2	−0.1	+2.6	+3.6	+2.3
Blood solids per cent one hour after return to ordinary room temperature	20.4	20.7	21.7	20.1	22.0	20.7	

$ Dog died half an hour after removal from hot room.
† Animal panted very vigorously during period in hot room.
‡ Moderate to intermittent panting during period in hot room.
‖ No panting.
* Small fan off. No air motion.

changed and blood solids showed increases of 0.2 and 0.1 per cent respectively, essentially unchanged.

Dilution level. Between 35.5° and 38.8°C., there was a moderate degree of panting, no significant change in body temperature, but a decrease in blood solids of 0.4 per cent in one case, and 0.9 per cent in the other case.

In the above two cases, as in those described under the condition of high relative humidity, there was also some degree of blood concentration in the after-period.

Transition level. This lies in the neighborhood of 42°C., thus in one experiment at this temperature the animal panted fairly vigorously but the temperature and blood solids were essentially unchanged, the animal apparently regulated perfectly by panting, the flow of water from the tissues to blood stream now being exactly compensated by evaporation.

Concentration level. At all observed temperatures between 42° and 54.5° C., there was very vigorous panting, a marked rise in body temperature and a considerable increase in blood solids, 2.8 per cent at 47°C., 3.1 per cent at 50°C., 2.7 per cent at 50°C. and 1.6 per cent at 54.5°C. Under these conditions there was also a loss of 200 to 300 grams in body weight. On return of the animal to ordinary room temperature body temperature rapidly fell to normal and coincidentally the blood solids tended to reach their normal level. (See fig. 2.)

The data from the low humidity experiments are set down in table 2.

THE TEMPERATURE AND BLOOD SOLIDS CURVES FOR "DRY" AND "MOIST" HEAT. Both groups of experiments are graphically summarized in figure 3.

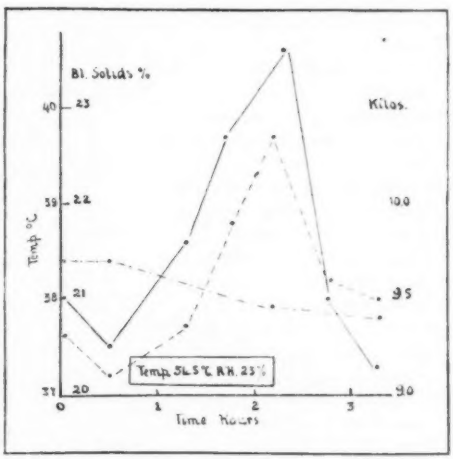


Fig. 2. Continuous lines, body temperature; broken lines, blood solids; dots and dashes, body weight.

In this chart changes in rectal temperature and blood solids percentage are plotted together against environmental temperatures. The effects recorded are those changes which occurred at the end of one hour's exposure to the room temperature indicated on the abscissa. Points below the abscissa indicate decrease, points above indicate increase.

In the "moist" air experiments it will be noticed that where the environmental temperature ranges between 27°C. and 33°C., the rectal temperature shows variations within normal limits. The blood, however, shows a dilution decreasing from 1.1 per cent at 27.8°C. to 0.1 per cent at 33°C., above these temperatures the rectal temperature rises rapidly in a practically poikilothermic curve, the blood solids percentage also mounts rapidly, 2.6 per cent at 37°C., 3.6 per cent at 40.5°C. and 2.3 per cent at 41°C.

In the "dry" air experiments it is apparent that between 33° and 39°C. the rectal temperature varies within normal limits, but the blood shows dilution, the blood solids showing a decrease of 0.9 per cent at 37.5°C. and of 0.4 per cent at 38.8°C., below these temperatures there is no change. Above 42°C. the rectal temperature rises, the increase varying in extent in different animals, the blood solids percentages also showing a marked increase.

TABLE 2

Effects of various room temperatures with low humidity (21 per cent to 51 per cent) on body temperature and blood solids of dogs

	EXPERIMENT NUMBER								
	27‡	28‡	8‡	7‡	6†	5†	23†	22†	26†
Temperature °C. of hot room	33.5	35.5	37.8	38.8	42.0	47.0	50.0	50.0	54.5
Normal temperature °C. of animal	37.8	37.9	38.4	39.6	38.7	38.1	38.5	38.8	37.5
Temperature °C. of animal after one hour's exposure in hot room	37.6	38.0	38.4	40.1	39.0	?	43.4	41.3	39.7
Net change in animal's temperature °C. after one hour's exposure in hot room	−0.2	+0.1	0	+0.5	+0.3		+4.9	+2.5	+2.2
Temperature °C. of animal one hour after return to ordinary room temperature °C.	37.6	38.5	38.4	38.7	38.1	?	38.5	37.3	37.3
Normal blood solids per cent	21.0	22.0	20.5	21.4	21.1	19.8	20.4	20.3	20.2
Blood solids after one hour's exposure in hot room	21.2	22.1	19.6	20.9	21.3	22.6	23.5	23.0	21.8
Net change in blood solids after one hour's exposure in hot room	+0.2	+0.1	−0.9	−0.4	+0.2	+2.8	+3.1	+2.7	+1.6
Blood solids one hour after return to ordinary room temperature	20.8	22.7	20.3	21.2	19.8	24.9	21.1	21.1	21.0

† Animal panted very vigorously during period in hot room.
‡ Moderate to intermittent panting during period in hot room.

In one experiment the humidity fell midway between the "dry" and "moist" ranges, the dry bulb reading being 39.2°C. and the relative humidity 63 per cent. Here the rectal temperature was found to increase by 1.1°C. and the blood solids by 1.6 per cent. The position of these points on the chart (denoted by the asterisks) lies, as would be expected, between the "moist" and "dry" curves.[2]

[2] The temperature of this dog after two hours' exposure was increased 1.2°C., $\frac{1}{10}$ of a degree higher than shown in the chart, which is the effect after one hour. This is of interest in connection with six experiments of Haldane (4) in 1905 on man

Comparing the temperature curves in the "moist" air and that in the "dry" air, it is obvious that in the former practically no regulation at all takes place in room temperature above 33°C., while in the latter regulation disappears more gradually and at a much higher temperature.

Discussion. The channels through which a dog may lose heat under moderate conditions of temperature are radiation and conduction from the

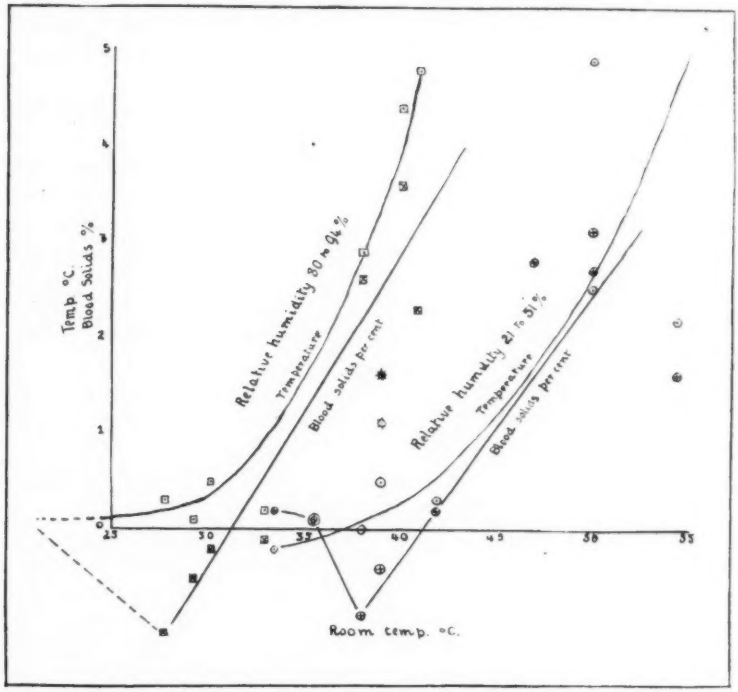


Fig. 3. ⊠ Blood solids per cent, ⊡ Rectal temperature } High relative humidity

⊕ Blood solids per cent, ⊙ Rectal temperature } Low relative humidity

made at the same degree of humidity (61 per cent to 67 per cent). These subjects were at rest in a room in which the dry bulb temperature varied between 38.9°C. and 40°C. The rise in rectal temperature after two hours' exposure would if plotted on our chart fall close to the region of the dog experiment just cited. This shows that the reaction of both man and dog are closely parallel under the given condition, if one assumes that 10 kilo dogs will be affected twice as rapidly as man.

body surface and some evaporation from respiratory tract and the mucous membrane of the mouth. Under more severe conditions polypnea comes into play, when the dog will give off much more heat by evaporation.

Our experiments were directed toward closing one or more of these avenues as desired. Thus, first, where heat dissipation by radiation and conduction was to be prevented the room temperature was raised above that of the animal's body temperature; secondly, where heat dissipation by evaporation was to be reduced to a minimum, the air was very highly charged with moisture; and thirdly, where all avenues of heat dissipation were to be closed the two measures were combined.

METHODS OF HEAT REGULATION IN THE PRESENCE OF THESE ARTIFICIALLY PRODUCED CONDITIONS. At environmental temperatures from 22° up to 33°C. with low relative humidity, and "moist" air temperatures up to 24°C. the animal is not called upon to make any extensive efforts to regulate its body temperature. When the temperature is much below these limits, i.e., when cold, Barbour and Tolstoi have shown that the animal, in part, at least, regulates by blood concentration. Above the limits mentioned a temperature level (about 38° for dry, 28° for "wet" air) is reached which closely approximates the animal's own temperature, and were not regulation instituted, body temperature would rise by virtue of its own metabolism. Our experiments tend to show that under these conditions, the animal regulates, in part, by blood dilution, by which means peripheral blood flow and hence heat dissipation by radiation and conduction are greatly facilitated. This confirms the similar observation of Barbour and Tolstoi on dogs placed in hot baths at from 40° to 42°C.

In man wearing ordinary clothing suitable for temperate climates, Hill, cited by Young (5), has shown that the "skin-shirt" temperature closely approximates the conditions just described above. The observations of Kestner and collaborators, (cited in (3)) that exercise with sweating dilutes the blood, indicates a reaction parallel to that we have observed in dogs.

When the environmental air temperature rises appreciably above body temperature, 42°C. or higher, the body temperature rises and the blood concentrates.

Does this blood concentration at high temperature levels represent only the effect of removal of water from the blood by evaporation, or is it due, in a measure, to some protective mechanism which tends to shift water from the blood to the tissues and so reduce the amount of fluid exposed to the hot external environment? We know that the animal as a whole does not become dried out, and retains in fact a surplus of water, for the blood solids tend to return to normal with the temperature after the animal is removed from the hot room. This indicates that the blood concentration is not necessarily passive and further evidence may be adduced from the work of certain investigators on man from two points of view.

On the one hand great loss of fluid need not deplete the blood of water, thus Hunt (6) has shown that a man may lose as much as 2.5 kilograms through sweating and yet not alter the fluid content of the blood. His subject was exposed to "dry" heat varying between 60°C. and 84°C. No record of body temperature was taken, but by the hemoglobin method no change in blood concentration was evident. On the other hand, considerable loss of water from the blood may occur with relatively little dehydration of the body as a whole. Young, Breinl, Harris and Osborne have shown that the blood concentrated in men who were exposed to high temperatures with high relative humidity and hence where evaporation from the body was limited.

Their subjects were exposed naked in a hot room the dry bulb temperature of which varied from 34.4° to 41.6°C. the wet bulb temperature from 32.8° to 37.9°C. and the relative humidity from 66 to 90 per cent. Exposure for 35 minutes of a 67-kilo subject to a dry bulb temperature of 41.6°C. and a wet bulb temperature of 37.9°C. (relative humidity 76 per cent) resulted in a loss of 1220 grams in body weight and an increase of 2.4 per cent in blood solids. The same subject exposed for 60 minutes to a dry bulb temperature of 34.4°C. and a wet bulb temperature of 32.8°C. (relative humidity 90 per cent) lost 595 grams in body weight and the blood solids increased 1.0 per cent.

A second subject weighing 45 kilos exposed for 45 minutes to a dry bulb temperature of 39.4°C. and a wet bulb temperature of 33.1°C. (relative humidity of 66 per cent) experienced a loss of 506 grams in body weight and an increase in blood solids of 2.1 per cent.

A third subject weighing 89 kilos exposed for one hour to a dry bulb temperature of 38.4°C. and a wet bulb temperature 34.2°C. (relative humidity 74 per cent) resulted in a loss of 970 grams in body weight and an increase in blood solids of 3.1 per cent.

It is difficult to believe that the body weight losses in these experiments represent more than a fraction of the water reserve, and yet the blood became highly concentrated.

Why then do not dogs, like Hunt's man, when exposed to "dry" heat, also maintain constant blood concentration? Considering that the evaporating surface of man is both relatively and absolutely many times greater than that of dogs, it may be that the animal concentrates its blood because it needs further means of protection against heat.

Since the animal in the hot chamber loses weight by evaporation of water, proof that the observed blood concentration is due to something besides loss of water from the blood to the air is still needed. It was hoped that by saturating the air of the hot room with moisture the animal could be prevented from losing water by evaporation. This, however, we were unable to accomplish since even when the moisture was condensing on the

walls of the cabinet the animal was still able to lose weight by blowing off water, so that whether or not the blood concentration in dogs at high temperatures is due to actual shifting of water from blood to tissues or mere dehydration of the blood still remains to be definitely determined.

CONCLUSIONS

1. In "moist" air temperatures between 27° and 30°C. and in "dry" air temperatures between 37° and 39°C. dogs, in a measure, regulate body temperature by blood dilution.

2. In "moist" air temperatures above 33°C. dogs are unable to regulate body temperature, the animal becoming practically poikilothermic. Under these conditions marked blood concentration occurs.

3. In "dry" air temperature above 42°C. dogs regulate body temperature very imperfectly, the body temperature rises with marked concentration of the blood.

4. It is suggested that the blood concentration observed in 2 and 3 may be ascribed in part to a protective mechanism which drives water into the tissues, rather than merely to excessive blood dehydration. The only positive evidence for this, however, is that the tissues are not dehydrated simultaneously with the blood.

I am pleased to express to Prof. H. G. Barbour, who suggested this research and supervised its execution, my deep sense of gratitude for his interest and encouragement.

BIBLIOGRAPHY

(1) Barbour and Tolstoi: This Journal, 1922, lix, 488; Ibid., 1923, lxvii, 378.
(2) Young, Breinl, Harris and Osborne: Proc. Roy. Soc., London, 1920, xciB, 111.
(3) Barbour: Physiol. Reviews, 1921, i, 295.
(4) Haldane: Journ. Hygiene, 1921, xii, 494.
(5) Young: Ann. Trop. Med. and Parasit., xiii, 313.
(6) Hunt: Journ. Hygiene, 1921, xii, 479.

HEAT REGULATION AND WATER EXCHANGE

IV. The Influence of Ether in Dogs[1]

HENRY G. BARBOUR and WESLEY BOURNE

From the Department of Pharmacology, McGill University

Received for publication September 24, 1923

This work was undertaken to study the part played by water shifting in the body in the response of the heat-regulating mechanism to changes in environment during anesthesia. The present paper is concerned entirely with ether.

While it has long been known that anesthetics exhibit a tendency to make animals poikilothermic (examples are cited in a recent review of heat regulation (1)) we are still in ignorance of the precise conditions of environment which are optimum for maintaining a normal body temperature under the influence of ether. Furthermore, little has been discovered as regards the concentration of the blood during anesthesia, or how its alteration may concern the welfare of the subject.

The concentration of the blood has indeed been studied in a large number of clinical cases by observations on hemoglobin and red cell count, but the results have been conflicting owing to the difficulties which clinical conditions present for proper control. For a review of this subject one may refer to the paper by Casto (2). On dogs, however, Hamburger and Ewing (3) found during ether anesthesia an increase of 10 per cent in the hemoglobin and 16 per cent in the red cell count. These changes did not entirely disappear under their conditions within six days. Acute increases of this sort in the solid constituents of blood are presumably to be interpreted as due mainly to a loss of water.

One of us has already brought evidence that the normal exchange of water in the response to changes in environmental temperature is interfered with by anesthesia. In the first paper of this series (4) it was shown that a dog under morphine and chloretone does not give responses to external cold and heat which are typical of the normal animal. Concentration of the blood from the cold was seen in one such animal, but under deep anesthesia no changes in the blood solids were obtained.

[1] A part of the results here described was reported before the Canadian Society of Anesthetists, at Montreal, June 12, 1923.

The quantitative changes in the temperature and blood solids of dogs in response to varying degrees of high temperature in wet and dry atmospheres have been worked out by Doctor Lozinsky in this laboratory. In the preceding paper (5) he showed a maximum dilution of the blood at about 28° in moist atmospheres, and 38° in dry. This was converted into concentration of the blood in each instance at temperatures a few degrees higher, along with the beginning of the breaking down of the heat regulating mechanism.

The experiments reported below have been made under precisely similar conditions using, however, the etherized instead of the normal dog. They will be reported in two series. The first includes hot chamber experiments with ether alone, while the second series shows how such experiments are influenced by the pre-anesthetic injection of fluids subcutaneously. The water exchange in this work was, however, studied with reference not only to the concentration of the blood but also to the amount of water excreted by the kidneys and the respiratory passages. This was done especially for the purpose of giving an idea of the heat loss by evaporation, and incidentally information concerning secretions was acquired.

Methods. The dogs were exposed to high temperatures by placing them in the large constant-temperature chamber described in Doctor Lozinsky's paper. Most of the experiments were done in dry atmospheres. In all of three moist experiments the results varied so little from those under the dry conditions that it was considered unnecessary to attempt to distinguish the effects of water vapor at the present time.

After finding that the optimum temperature for etherized fasted dogs was approximately 31°C. this temperature was made one of the standard conditions for the succeeding experiments. In addition to the wet-bulb and dry-bulb, readings were now made also with the kata-thermometer, in each experiment the instrument being placed within about 18 inches of the animal's head. In this part of the chamber the air could be described as "gently fanned." The small fan used to keep the air circulating was operated in one of the opposite corners of the room, where the dry and wet bulb readings were taken. The motor used for this purpose was placed outside the chamber, interference by radiation of heat from the motor was thus abolished.

All dogs used were healthy females which had been fasted for at least forty hours before the experiment with water allowed in each case up to the time of beginning.

Each experiment was begun with a fore-period of an hour or more during which the dog was kept at room temperature. The ether period which followed was extended over two hours during the first part of the work; this was reduced to one hour when it was established that definite changes

of significance did not occur during the second hour. Before and after the experiment and between the two periods the following procedures were carried out: Washing out of the bladder with determination of the amount of urine; weighing the dog to the nearest 5 grams; the taking of duplicate samples of the blood from the saphenous vein above the ankle; and the reading of the (8 cm. deep) rectal temperature. During the ether period the actively secreted saliva was also collected and weighed.

Ether anesthesia was induced in a large glass box (with wooden frame and floor) 13 × 28 × 19 inches, within the incubating room. Ether was blown through this box from outside of the chamber. As soon as the dog had reached the first stratum of the third stage of anesthesia it was removed to a table in the same incubating room, and anesthesia was continued through the same tube intrapharyngeally throughout the remainder of the experiment. By observation through the triple glass door, the same depth of anesthesia was maintained constantly throughout the experiment.

Ether induction always consumed approximately five minutes; before this and immediately thereafter the box was weighed, the result usually representing saliva, although where noted in the tables, sometimes including feces. (The wooden frame of the box was shellaced both inside and out.) During the balance of the ether period the saliva was collected and ether weighed on tared cotton or measured in a cylinder.

Results. The effects of ether anesthesia in dogs at various constant room temperatures from 20° to 37°C., moist and dry, are summarized in table 1. The first two experiments included were not done in the constant temperature chamber but in a laboratory room without special air motion. From 25° to 30.5°C. one sees a progressive diminution in the fall of body temperature noted in the first hour after anesthesia, while from 31°C. upward the increases became progressively greater. The only experiment in which the temperature remained unchanged was that at 30.5°C. One is somewhat surprised at first to see that the results in the moist chamber correspond closely with those in the dry, but this is taken simply as an indication that the animal is entirely without the power of regulation against either heat or cold in both cases.

The results on body temperature plotted against the different incubator temperatures employed, will be seen in figure 1, where they are represented by the heavy dots. For comparisons with normal conditons the smoothed curves from Doctor Lozinsky's paper are reproduced in this same figure. As regards regulation against over-heating it will be seen that all of the determinations in etherized dogs, moist or dry, lie between the dry curve and the moist curve for the normal dog, but close to the latter. This position appears to be determined by the following facts. The normal dog in a saturated atmosphere is practically helpless to regulate

TABLE 1

Effects of ether anethesia at various room temperatures (moist and dry)

	EXPERIMENT NUMBER										
	1*	2*	5	9	6	7	4	11	10	3	8
Temperature °C. of hot room (Dry bulb)	20.8*	25.0*	25.0	26.5	27.5	30.0	30.5	31.0	31.5	35.5	37.0
Temperature °C. of hot room (Wet bulb)			17.0	25.5	19.5	24.5	23.5	29.5	25.5	27.5	34.0
Relative humidity per cent	Low	Low	45	93	47	64	55	89	61	54	81
Normal temperature °C. of animal	38.5	38.2	38.6	38.6	38.8	38.85	38.9	39.05	38.9	38.35	38.7
Temperature °C. of animal ofter one hour's exposure in hot room	37.5**	36.8	36.8	37.55	37.55	38.15	38.9	39.35	39.35	39.0	39.8
Net change in animal's temperature °C. after one hour's exposure in hot room	−1.0**	−1.4	−1.8	−1.05	−1.25	−0.70	0	+0.30	+0.45	+0.65	+1.1
Temperature °C. of animal after two hours in hot room	36.4	36.8	34.85	35.95	35.55	35.0	39.1	39.15	40.0	39.2	40.9
Normal blood solids per cent	20.0	21.5	22.0	22.0	22.2	22.9	23.3	18.5	22.0	20.9	19.7
Blood solids per cent after one hour's exposure in hot room.	23.3	23.4	25.0	25.3	25.6	25.6	25.4	21.2	25.4	23.6	22.2
Net change in blood solids per cent after one hour's exposure in hot room	+3.3	+1.9	+3.0	+3.3	+3.4	+2.7	+2.1	+2.7	+3.4	+3.3	+2.5
Blood solids per cent after two hours in hot room	23.3	23.6	25.3	24.4	25.1	24.8	25.3	21.0	24.5	24.3	22.1
Water expired per hour (calculated)			20.83		25.24		33.72	30.4	32.1	56.8	

*Laboratory room, no breeze.

**Estimated from 2 hour period.

against higher environmental temperature than about 28°C.; the etherized dog is equally powerless, but owing to a somewhat lowered heat production, withstands a slightly higher temperature (31°C.). As regards the

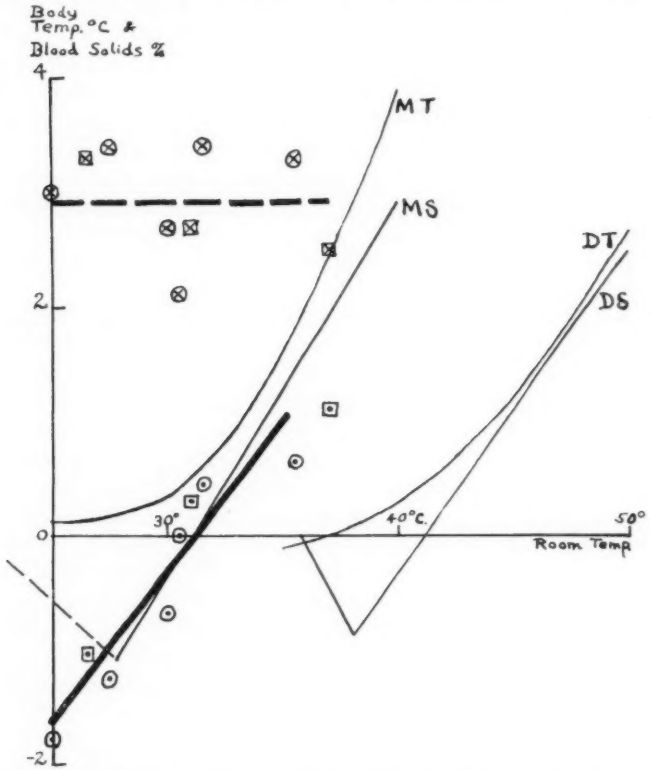


Fig. 1. Effects of ether upon temperature and blood solids of dogs exposed to high temperatures. Each point represents a change from normal produced by the first hour's exposure to the constant temperature indicated by the abscissa. Light lines represent smooth curves from Doctor Lozinsky's work on normal dogs: *MT:* curve of body temperature change at various incubator temperatures *moist; MS:* curve of blood solids change at various incubator temperatures *moist; DT:* curve of body temperature change at various incubator temperatures *dry; DS:* curve of blood solids change at various incubator temperatures *dry;* ⊙: body temperature change under ether; *dry* environent; ⊡: body temperature change under ether; *moist* environment. Heavy continuous line: smoothed temperature curve; ⊗: blood solids change under ether; *dry* environment; ⊠: blood solids change under ether; *moist* environment. Heavy broken line: mean level of blood solids for all experiments.

cold, of course, the regulation in normal dogs breaks down only at far lower air temperatures than those under consideration here. The ether-

ized dog, however, shows a continuation of the body temperature curve straight down below the optimum point at the same rate as it rises above this.

The effects of ether upon the blood solids are of the same magnitude as the changes found by Hamburger and Ewing in the hemoglobin and red cell count at ordinary temperatures. Under our conditions room temperatures varying from 25° to 37°C. did not alter this response. These ranged from plus 2.1 per cent to plus 3.4 per cent irrespective of incubator temperature. The individual results are also plotted in figure 1, the heavy broken line indicating the average result (2.93 per cent).

Blood concentration occurs then at an environmental temperature range where normal dogs in dry atmospheres remain unaffected, but in moist atmospheres exhibit a curve passing from blood dilution to maximum concentration.

Interpreting these results on the blood solids on the basis of the previously advanced theory of heat regulation one can say that the etherized dog is quite unable to shift water in response to changes in environmental conditions. The stimulus of the ether itself is the all-powerful one as regards the condition of the blood. Ether may be classed among the hostile stimuli such as extreme cold, extreme heat, and various agents which have been demonstrated to stimulate the adrenal gland,[2] all producing concentration of the blood. Conceiving this response as an attempt at protection, we may say that ether like extreme cold and extreme heat tends to drive the water away from the surface of the body, making the outer tissues much freer from circulating blood and hence a much better insulator against cold and heat. As DuBois expresses it for fever patients, the outer tissues of the body are thus transformed into a suit of clothes. In the present case the protection is quite inadequate but it seems reasonable to assume that it has been attempted.

What is the fate of the water which is driven out of the blood under ether at the various temperatures? It is scarcely possible to account for the whole water reserve of the body by reference to that lost in urine, saliva and expired air, and it seems probable that much is driven into the tissues. The water balance from six of the experiments is summarized in table 2, which also includes a partial summary of three other experiments. From this it appears that the expired air contained an amount of water gradually increasing with the increase of environmental temperature (see also table 1, last line). The results are quite uniform although certain theoretical possibilities have not been completely reckoned with. (Among these are variability in loss of weight in the form of

[2] Hartman (7) has recently demonstrated in cats that cold baths stimulate the output of epinephrin, although, he believes, not sufficiently to account for the concentration of the blood.

CO_2, and in gain by ether.) Upon normal dogs exposed to similar environmental conditions we have at present no experiments; but so far as

TABLE 2

Water-loss in dogs etherized at various room temperatures

EXPERIMENT NUMBER	ROOM TEMPERATURE		DURATION	DOG'S WEIGHT	WEIGHT LOST				WATER LOSS PER HOUR	
					Total	Urine	Saliva, etc.	Respiration calc.	Respiration	Urine
	°C.		*hrs. min.*	*gms.*						
3		Before exper.		10,540						
	19.5	Before ether	1:0	10,495	45	31		14	14.0	31.0
	35.5	After ether	2:30	10,275	220	28	50	142	56.8	11.2
4		Before exper.		10,320						
	19.0	Before ether	2:25	10,270	50	30		20	8.2	12.3
	30.5	After ether	2:35	10,155	115	00	28	87	33.7	0
5		Before exper.		10,810						
	21.0	Before ether	1:10	10,760	50	31		19	16.2	26.4
	25.0	After ether	2:10	10,610	150	19	86*	45	20.7	8.7
6		Before exper.		10,700						
	20.0	Before ether	1:5	10,670	30	9		21	19.4	8.3
	27.5	After ether	2:25	10,490	180	5	114*	61	25.2	2.0
7		Before exper.		10,540						
	24.0	Before ether	1:15	10,535	5	8	—	—		6.4
	30.0	After ether	2:20	10,370	165	2			—	0.9
8		Before exper.		9,915						
	22.0	Before ether	1:20	9,890	25	13	—	—	—	9.7
	37.0	After ether	2:20	9,760	130	4	—	—		1.7
9		Before exper.		10,500						
	22.5	Before ether	1:7	10,480	20	4			—	3.5
	26.5	After ether	2:23	10,300	180	1	—	—		0.4
10		Before exper.		11,195						
	25.0	Before ether	1:24	11,145	50	20		30	21.8	14.5
	31.5	After ether	2:18	11,005	140	2	64	74	32.1	0.8
11		Before exper.		17,240						
	27.0	Before ether	1:50	17,145	85	16		69	37.7	8.7
	31.0	After ether	2:30	16,975	170	4	90	76	30.4	1.6

*Including feces.

can be judged our results show no influence of ether. For example, they accord well with the average water output of the lung in the pre-anesthetic periods when allowance is made for room temperature.

As regards the excretion of urine, the oliguria usually described under ether is clearly seen; complete anuria occurred in only one case, but the reduction in urine flow seen in the other cases amounted to from about 67 to 95 per cent.

The results on the blood concentration suggest, however, an explanation for ether anuria which we have never seen advanced previously, namely, that it is due to lack of available water in the blood rather than to any effect upon the kidney itself.

TABLE 3

Effects of ether anesthesia at 30° to 31°C. dry air after fluid injections

	EXPERIMENT NUMBER					
	13	15	16	18	17	12
	Fluid injected (subcutaneously)*					
	H_2O	H_2O	3 per cent dextrose	3 per cent dextrose	5 per cent dextrose	5 per cent dextrose in Locke
Temperature of hot room °C. Dry bulb.	30.5	30	31	30	31	31
Temperature of hot room °C. Wet bulb.	23	21.5	22.5	20	22.5	22.5
Relative humidity	53	47	48	39	48	48
Kata-thermometer (millicalories per sq. cm. per sec.) Dry..			1.5	1.8	1.6	1.8
Kata-thermometer (millicalories per sq. cm. per sec.) Wet..			11.2	13.6	11.0	12.8
Temperature °C. of animal before fluid	39.0	38.1	39.3	39.15	39.3	38.95
Temperature °C. of animal after fluid	39.55	37.8	39.9	38.55	39.85	39.0
Temperature °C. of animal after one hour in hot room	38.65	37.0	37.8	37.1	37.8	38.7
Net change while in hot room	−0.9	−0.8	−2.1	−1.45	−2.05	−0.3
Blood solids per cent before fluid	19.5	21.1	20.2	21.6	19.2	18.8
Blood solids per cent after fluid	18.0	20.6	20.9	23.9	20.7	19.5
Blood solids per cent after one hour in hot room	19.5	21.6	21.2	23.2	21.9	23.3
Net change while in hot room	+1.5	+1.0	+0.3	−0.7	+1.2	+3.8
Water expired per hour (calculated)	0 ?	0 ?	12	0 ?	20	11.1

*Amounts in table 4.

RESULTS OF SUBCUTANEOUS INJECTIONS OF FLUID BEFORE OR DURING THE FORE-PERIOD. An obvious outcome of the work reported up to this point was to determine whether and to what extent the concentration of the blood could be influenced by preliminary provision for fluid subcutaneously. To this end we made a second series of experiments for which were adopted standard experimental conditions previously found to approach the optimum for the etherized dog. These were a temperature of 30° to 31°C. and as dry air as possible. The relative humidity

varied from 39 per cent to 63 per cent in the six experiments. In four of these our conditions were checked by the kata-thermometer (6)[3] (table 3). A very low cooling power of the environment was indicated,

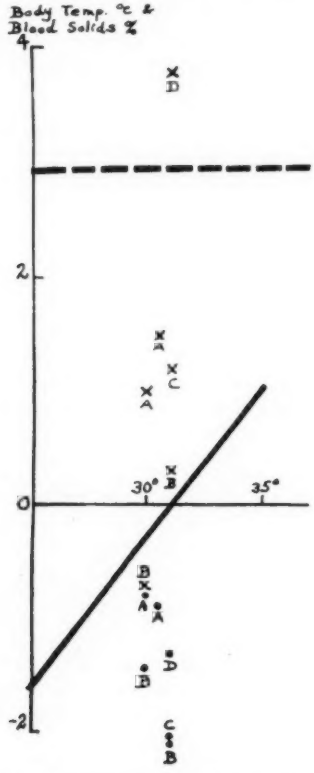


Fig. 2. Effects of first hour's exposure to ether after preliminary subcutaneous injections of fluid.

●: Body temperature
×: Blood solids
} change under ether {
A after dist. H_2O
B after dextrose 3 per cent
C after dextrose 5 per cent
D after dextrose 5 per cent in Locke's solution

Heavy lines reproduced from figure 1 to show average effects of ether alone: continuous line, body temperature; broken line, blood solids.

especially with the dry bulb (1.5 to 1.8 millicalories per sq. cm. per second). The wet bulb gave from 11.0 to 13.6 millicalories per sq. cm. per second.

[3] We desire to thank Prof. A. Norman Shaw of the Physics Department for the use of his instrument and the benefit of his experience with it.

The optimum "dry" and "wet kata cooling power" for dogs under ether lie therefore at about 2 and 12 respectively.

Under the above conditions ether was administered following the subcutaneous injection of about one liter of fluid. In two experiments distilled water alone was injected, in two 3 per cent dextrose, and in one each 5 per cent dextrose in water and Locke's solution respectively. The sum-

TABLE 4

Water-loss in dogs etherized at 30 to 31°C. after fluid injections

FLUID INJECTION	EXPERIMENT NUMBER		DURATION	WEIGHT	WEIGHT LOST				WATER LOSS PER HOUR	
					Total	Urine	Saliva	Respiration calc.	Respiration	Urine
			hrs. min.	*gms.*	*gms*	*cc.*	*cc.*	*gms.*	*gms.*	*cc.*
5 per cent dextrose in Locke, 1345 grams	12	Before exper.		*18,215						
		Before ether	0:50	18,155	60	10	—	50	60.1	12.0
		After ether	2:20	18,020	135	19	90	26	11.1	8.1
Distilled H_2O, 924 grams	13	Before exper.		*17,464						
		Before ether	1:10	17,350	114	6	—	108	92.3	5.1
		After ether	1:10	17,250	100	6	95	0?	0?	5.1
Distilled H_2O, 1200 grams	15	Before exper. (after H_2O)		9,515						
		Before ether	1:30	9,480	35	00	—	35	23.3	0.0
		After ether	1:15	9,420	60	2	71	0?	0?	1.6
3 per cent dextrose 1,000 grams	16	Before exper.		8,960						
		Before ether	1:0	8,920	40	1	—	39	39.0	1.0
		After ether	1:5	8,830	90	00	77	13	12.0	0.0
5 per cent dextrose 1,000 grams	17	Before exper.		8,850						
		Before ether	0:55	8,815	35	17	—	18	19.5	18.0
		After ether	1:15	8,760	55	00	30	25	20.0	0.0
3 per cent dextrose 1,000 grams	18	Before exper.		8,210						
		Before ether	1:5	8,155	55	6	—	49	45.3	5.5
		After ether	1:5	8,125	30	00	32	0?	0?	0.0

*Corrected for fluid injected just before weighing.

mary in table 3, as well as the lettered points in figure 2, present the results. The change in blood solids produced by ether was much reduced by every one of these injections. The greatest influence exerted, however, was by the 3 per cent dextrose, which is hypotonic. This caused a limitation of the ether effect to an increase of 0.3 per cent in one case, while

in the other the blood solids were actually reduced by 0.7 per cent during the ether period.

Body temperature in every case also showed a considerable reduction, although the dogs etherized without fluid injections had exhibited little or no change in temperature under the environmental conditions chosen. This may be tentatively attributed to the dilution of the blood promoting radiation from the surface of the animal, in accord with the idea that ether concentrates the blood in order to protect against radiation.

It is further worthy of note that distilled water promotes a tendency to concentration more than does 3 per cent dextrose, similarly it causes a somewhat lesser degree of temperature loss. These facts are attributed to the irritating properties of distilled water and class it among the hostile stimuli previously referred to, insofar as its effect upon cutaneous and subcutaneous nerve endings is concerned.

The other data relating to the water balance are presented in table 4, but are not considered sufficiently complete to warrant final deductions. One sees, however, that the respiratory water loss was much reduced where fluid was injected. This is probably largely due to the quieter respirations uniformly observed.

Concerning the renal excretion of water the data are also incomplete, but in only two experiments of the six is any indication of the usual inhibition produced by ether seen. The smaller dogs used in this series, however, gave a disappointingly low normal flow. In general it appears that the oliguria which usually results from ether can be prevented by a sufficient supply of water. This supports the idea that it is due to blood concentration rather than renal inhibition.

Perhaps the most important practical deduction from the second experimental series is that if it is desirable to maintain a normal blood concentration under ether this may be done by prophylactic fluid injections, but the use of these renders necessary a further reduction in the cooling power of the environment if body temperature is to be kept normal.

CONCLUSIONS

1. Normal dogs under ether anesthesia are so lacking in power to regulate their body temperature that it remains constant only at an environmental temperature very near 31°C. in either wet or dry (gently fanned) atmospheres.

2. Irrespective of environmental conditions ether always concentrates the blood, the blood solids increasing by 2 to 3.5 per cent of the total weight of the blood. This increase is probably not due to water loss from the body, but water appears to be driven out of the blood by a

reflex from ether irritation as by other "hostile stimuli" (as extreme heat or cold, emotions, etc.).

3. The expired water during ether anesthesia does not appear to be abnormal in amount.

4. Pre-anesthetic injection of fluids (hyper- and hypotonic dextrose solutions and distilled water) reduces markedly the blood concentration produced by ether given in a dry environment of 30° to 31°C.; in fact, 3 per cent dextrose seems to prevent it entirely. This failure to concentrate the blood appears to account for the lower body temperatures these fluid injections produce. The blood concentration after ether for this and other reasons appears to represent an attempt at protection against environment.

5. Ether oliguria and anuria are probably due more to thickening of the blood than to any renal factor.

6. For etherized dogs the optimum "dry" and "wet kata cooling power" of the environment lies near 2 and 12 millicalories respectively. It lies still lower when prophylactic fluid injections are employed.

BIBLIOGRAPHY

(1) Barbour: Physiol. Rev., i, 1921, 320.
(2) Casto: American year book of anesthesia and analgesia, 1915.
(3) Hamburger and Ewing: Journ. Amer. Med. Assoc., 1907, li, 31.
(4) Barbour: This Journal, 1924, lxvii, 366.
(5) Lozinsky: This Journal, 1924, lxvii, 388.
(6) Hill: Medical Research Council, Special Report Series 1919–23, nos. 32, 52, 73.
(7) Hartman and Hartman: This Journal, 1923, lxv, 623.

THE EFFECTS OF EXTREME TEMPERATURES ON FISHES

S. W. BRITTON

From the Atlantic Biological Station, St. Andrews, N. B.

Received for publication August 20, 1923

A number of observations are on record with regard to the ability of fishes to withstand various extreme degrees of temperature. John Hunter (1), for example, found that the body temperature of an eel could be reduced to 0.6°, and that although at first it appeared to be dead, recovery took place when the surrounding temperature was raised. According to Regnard (2), carp may recover after immersion in water (containing 2.5 per cent magnesium sulphate) at two or three degrees below zero. It is also stated by Pictet (3) that fishes may be revived after freezing until they are as brittle as ice. In contrast with these results a very hardy fish, *Fundulus heteroclitus*, (the common mummichog) used by Martin (4) in low-temperature experiments, was shown to survive a temperature of −1.0° for only a very short time.

Concerning the effects of the exposure of fishes to high temperatures, no definite information is available, although travellers and explorers have on various occasions reported the observation of fish actively swimming about in the hot water of thermal springs.

The experiments considered in the present paper were carried out in the laboratories of the Canadian Government Atlantic Biological Station at St. Andrew's, New Brunswick. The purpose of the inquiry was to determine with precision the higher and lower temperature limits at which widely varying types of fishes, such as the flounder, cod, eel, skate, etc., may still maintain life, and to observe the effects of various extreme temperatures on heart and respiratory rates, on reflex action and on general behavior.

According to the size and quantity of the material available, from one to five fishes, recently caught either by net or by hook, and generally ranging between one-half and five pounds, were employed in an experiment at one time.

LOW TEMPERATURE EXPERIMENTS. The low-temperature apparatus consisted of a large, well-insulated, double-walled tank, around the inner compartment of which a brine mixture of any desirable temperature could be maintained. An adjustable framed-glass cover allowed observations to be made during the progress of an experiment.

The sea-water used (that found in the neighborhood of the mouth of the St. Croix River) commenced to freeze at a temperature of $-1.6°$; it was observed, however, that after about one-quarter of the sea-water in the cooling tank had frozen, the temperature of the remaining water (now at a higher concentration because of the separation of ice in freezing) fell to a limit of $-1.9°$. It was found that the greater number of fishes withstood exposure to a water temperature of $-1.9°$ for only a comparatively short while and, with one exception, lower temperatures are therefore not considered in the present article.

Two methods of subjecting fishes to low temperatures were employed, which may be called "slow" and "rapid" respectively: in the former case the water temperature of the cooling tank into which the fish had already been introduced was slowly reduced from about $10°$ to $-1.9°$; in the latter case, the fishes were at once transferred from the stock tank (water temperature $12°$ to $16°$) to the cooling tank, the temperature of which had previously been reduced to about zero. In each case the end result, i.e., the ability of a fish to withstand the lowest freezing temperature of the water, was found to be approximately the same.

Spontaneous respiratory movements of teleosts were arrested in 5 to 30 minutes when they were exposed to a temperature of $-1.9°$; on the other hand, elasmobranchs frequently maintained very slow and shallow breathing from one to two hours or more after such exposure. It was observed that in all cases jaw, respiratory and several fin reflexes persisted for a variable time after the subsidence of respiration.

The usual procedure followed in the earlier experiments was to withdraw the fish from its extremely cold environment at the end of successively greater intervals of time after the cessation of respiration, or (later) after the disappearance of reflexes, and to replace it in the stock tank; hence, from the relative rapidity or absence of recovery a fairly accurate measure of its power of resistance to cold was obtained. In later experiments, fishes which had given indication of a high degree of resistance were maintained in the cooling tank over night.

The following data are abstracted from the later low-temperature experiments:

Experiment 20. July 27, 1922.

Fishes 1 and 2—Sea raven (Hemitripterus americanus)—4 pounds, 10 ounces and 2 pounds, 6 ounces respectively.

TIME	WATER TEMPERATURE	RESPIRATION PER MINUTE		NOTES
		Fish 1	Fish 2	
p.m.				
4:10	16.9°	21	27	Fishes in stock tank, quiescent at bottom; transferred to cooling tank
4:15	7.8	20	11	
4:30	6.5	14	10	Both continually swimming around tank; respiration deep and regular
4:45	3.3	11	8	
5:30	0.3	7	5	Respiration very deep; rate increased on touching pectoral region. Both fish quieter but easily stimulated to vigorous movement on tapping any part of body
6:10	−0.4	5	3	
7:00	−1.5	(?)	1–2	No. 2 respiring irregularly and less deeply; both fish extremely sensitive to stimulation such as stroking or tapping body with glass rod
8:00	−1.9	0	0	No cardiac or respiratory movements discernible. Gill covers extended, gills widely separated. No. 1 shows loss of equilibrium
11:00	1.4	0	(?)	Dorsal and caudal fin reflexes present in no. 2. Left both in cooling tank over night
July 28 *a.m.*				
8:15	4.6	0	11	No. 1 showed no spontaneous movement and was inexcitable to stimulation; on exposing heart (9 a.m.) it was found to be stopped, but contracted on stimulation (tapping). No. 2 respiring regularly and appears to be in good condition. Put in stock tank (water temperature 14.7°); respiratory rate rose to 22 per minute within three hours. Fish lived five days

Experiment 27. August 2, 1922.

Fishes 1, 2 and 3—Skate (R. ocellata)—2 pounds, 2 pounds 2 ounces and 1 pound respectively. Fish 4—Skate (R. radiata)—3 pounds 2 ounces.

7:00 p.m. All the fish in stock tank; water temperature 16.3°. Respiratory rates varied between 25 and 35 per minute, and heart rates between 19 and 23 per minute. All withdrawn and placed in low-temperature tank at −1.9°. Respiratory rates were appreciably slowed within the first 15 minutes of exposure, all the fish meanwhile showing vigorous swimming movements.

8:30 p.m. All quiescent; no respiratory movements except on stimulation, e.g., tapping in pectoral region; pseudobranchs widely extended. On withdrawal for examination, jaw, pectoral and pelvic fin, and caudal reflexes were easily elicitable. All replaced in tank (recharged with cooling brine surrounding) and left over night.

August 3, 9:00 a.m. Cooling tank temperature −1.9°; none of the fish show any signs of life. All withdrawn and placed on observation table in supine position. No spontaneous movements and no respiration could be detected. The pelvic fin reflex (on tapping in cloacal region) was present, but very slow in initiation and subsidence, in nos. 1 and 2; the apex beat in both fishes was observed over pectoral region, the rate in each case being three per minute with an apparent time interval of 1.5 to 2 seconds between the auricular and ventricular systoles. No branchial or cardiac movements and no reflexes were present in nos. 3 and 4; on opening pericardial cavities, heart in each was found to be stopped in diastole; single contractions of the whole heart were readily elicitable by touch.

9:30 a.m. Nos. 1 and 2 still on observation table. Heart rates 12 and 16 per minute respectively; jaw, pectoral and pelvic fin, and caudal reflexes present. Replaced both in stock tank; respiration recommenced a little later and good recovery was effected by each.

A number of fishes examined during the course of low-temperature experiments showed variations in deep rectal temperatures between −0.8° and 1.3°, the lower temperature being reached only after an animal had been kept in water at −1.9° for thirty minutes or more. In such an instance the caudal part or frequently the whole of the fish showed marked rigidity with maintenance of the body in the rectilinear position—as if, indeed, the animal had really been frozen stiff. This condition probably did not represent an actual congelation of the tissues, but rather a temporary cold-rigor, since eventual rapid recovery of the fish with no apparent disability occurred in the majority of cases under observation.

HIGH TEMPERATURE EXPERIMENTS. For the experiments which were carried out to determine the higher temperature limits compatible with life, a plain galvanized-iron tank of large capacity, with low-jet gas-heating apparatus arranged below, was provided. Uniform warming of the sea-water employed was ensured by the use of a stirring contrivance. The whole period of warming extended over approximately one hour, during which time the general and the particular behavior of a fish or fishes was studied, as in the low-temperature experiments.

Two high-temperature experiments are cited in brief:

Experiment 24. July 31, 1922.

Fishes 1, 2, 3 and 4—Sculpin (Myoxocephalus octodecimspinosus)—varying in weight between ¾ pound and 1½ pounds

TIME	WATER TEMPERATURE	RESPIRATION PER MINUTE				NOTES
		Fish 1	Fish 2	Fish 3	Fish 4	
a.m.						
10:30	14.3°	20	25	24	32	Fish in stock tank, all swimming about quietly; put in warming bath
10:45	17.8°	25	36	37	63	All restless and easily excited
11:00	21.2°	25	56	32	50	Nos. 1 and 3 respiring deeply. All extremely lively and attempt to jump from tank
11:20	25.4°	48	0	48	54	Loss of equilibrium and irregular asphyxial respiration in nos. 1, 3 and 4; spines elevated and gills widely extended. Fish 2 on cessation of respiration was withdrawn and replaced in stock tank
11:30	27.7°	0	0	0	0	Nos. 1, 3 and 4 stopped breathing; pectoral fin and spine reflexes (to touch) present, but each showed onset of rigor. Reduced temperature of bath to 16° in 8 to 10 minutes, then replaced all in stock tank at 14.3°
12:00	14.3°	0	0	0	23	Fish 4 showed asphyxial type of breathing for 10 minutes, followed gradually by quiet, regular respiration. In nos. 1, 2 and 3, all external reflexes were absent; on opening pericardial cavities, heart-beat was observed to persist regularly in all, at 16, 70 and 15 per minute respectively
p.m.						
2:00	14.8°				41	Nos. 1 and 3—heart rates 14 and 18 per minute respectively; later the beats became irregular, and eventually each heart stopped in diastole. No. 2—heart stopped, but stimulable to touch. No. 4— apparently normal. This fish lived 4 days in stock tank

Experiment 25. August 1, 1922.

Fishes 1, 2, 3 and 4—Flounders (Pseudopleuronectes americanus) varying in weight between 1 pound and 1½ pounds

TIME	WATER TEMPERATURE	RESPIRATIONS PER MINUTE				NOTES
		Fish 1	Fish 2	Fish 3	Fish 4	
a.m.						
11:15	14.7°	56	60	60	46	Fish in stock tank; withdrew and put in warming bath
11:30	18.3°	69	81	78	63	Respirations deeper and more rapid
11:45	22.5°	86	98	100	78	All the fish extremely active and swimming at the top of the water
12:00	27.8°	80	62	20	60	No. 3 turned over on its back and failed to recover the normal position. Breathing irregular and movements less vigorous in all
p.m.						
12:08	30.4°				25	No. 4 gasping; complete failure of respiration in others. All the fish showed the onset of rigor; gills widely distended. Replaced all in stock tank. No. 1 commenced spontaneous respiration within 3 minutes
12:30	14.9°	60			(?)	Fish 1 breathing regularly; no. 4 gasping; nos. 2 and 3 fail to show any recovery
12:45	14.9°	72				Fish 1 appeared normal; lived several days; others did not recover

In the greater number of experiments, when the temperature of the warming bath had reached 25° the fishes began to evince signs of distress, arhythmic or even asphyxial respiration most commonly occurring. The maintenance of the water at about this temperature level for some little time, however, apparently resulted in an adjustment of the respiratory mechanism to meet the demands of the new thermal condition, since perfectly rhythmic respiration at a faster rate was eventually established. If the warming process were pushed a few degrees farther, symptoms of respiratory and (later) of equilibratory distress became increasingly evident. It was thus observed that fishes could not be retained for many minutes in water at the higher temperatures (about 30°) without greatly endangering life.

TABLE OF EXPERIMENTS. The higher and lower limits of temperature sustained by different species of fish are given in table 1, derived from a series of over thirty experiments, in which nearly one hundred fishes were employed.

TABLE 1

NAME OF FISH	LOW TEMPERATURES (LOWEST TEMPERATURE OF WATER −1.9°)			HIGH TEMPERATURES		
	Time lowest temperature sustained	Number of fish used	Number of fish survived	Highest temperature of water	Number of fish used	Number of fish survived
Skate:						
Raja ocellata	4 h. 10 m.	1	1	28.4°	3	2
Raja ocellata	14 h. 0 m.	3	2	28.5	3	2
Raja ocellata	16 h. 0 m.	1	0			
Raja radiata	14 h. 0 m.	1	0			
Raja erinacea	16 h. 0 m.	2	0			
Sculpin:						
Myoxocephalus greenlandicus	15 m.	2	2	29.8°	4	0
Myoxocephalus greenlandicus	1 h. 10 m.	7	3	27.0	4	0
Myoxocephalus greenlandicus	2 h. 10 m.	7	0	27.1	2	1
Myoxocephalus octodecimspinosus	15 m.	4	2	29.8	1	0
Myoxocephalus octodecimspinosus	45 m.	4	1	28.9	2	1
Myoxocephalus octodecimspinosus	2 h. 10 m.	2	2	27.7	4	1
Sea raven:						
Hemitripterus americanus	20 m.	2	1	28.8°	3	1
Hemitripterus americanus	1 h. 0 m.	1	0			
Hemitripterus americanus	2 h. 30 m.	2	1			
Flounder:						
Pseudopleuronectes am.	50 m.	3	3	28.3°	2	1
Pseudopleuronectes am.	1 h. 15 m.	4	0	30.4	4	1
Pseudopleuronectes am.				31.4	4	2
Eel pout:						
Zoarces anguillaris	30 m.	1	0			
Zoarces anguillaris	2 h. 0 m.	1	1			
Cod:						
Gadus morrhua	30 m.	1	0			
Gadus morrhua	2 h. 0 m.	1	1			
Tomcod:						
Microgadus tomcod	5 m.	4	3	29.0°	3	3
Pollack:						
Pollachius virens	3 m.	1	1	27.9°	3	1

It is evident from the foregoing table that elasmobranchs have much greater ability than teleosts to withstand prolonged exposure to low temperatures. The higher temperatures, however, appear to be almost equally well borne by both classes of fishes.

The comparative inability of pollack and tomcod to sustain low temperatures well is possibly explained by the fact that the specimens obtainable were smaller and less mature than the other forms used.

None of the fishes were exposed to the highest water temperatures for any considerable period, and the time limits are therefore not quoted in the preceding table.

TEMPERATURE EFFECTS ON PULSE AND RESPIRATION. The heart-beat in the fishes used could after watchful practice be detected through pulsations of the body wall in either the pectoral or post-branchial regions.

Figures derived from a large number of fishes which were recently caught and kept in water at 11° to 15°, showed that the rate of the heart-beat under such fairly normal conditions averaged between 18 and 24 per minute; whilst on exposure for only a short period in the low temperature tank at −1.9° the rate fell to 4 or even 2 beats per minute. In some instances, on maintaining the fish in this latter temperature for an hour or so, no cardiac movement could be ascertained even after careful scrutiny, although regular contractions of the heart and ultimately full recovery of the animal were found to occur on elevation of the surrounding temperature.

In a special case in which a skate (*R. laevis*) was exposed for 16 hours in a refrigerator to a temperature as low as −20° (the whole of the fish being thereby solidly frozen and all reactions necessarily submerged), the heart-beat nevertheless returned to a slow and regular rhythm on gradual thawing of the animal, although the fish did not recover.

All the experiments have shown that, within limits, the ratio of the pulse rate to the surrounding temperature is a constant. This is illustrated along with the respiratory ratio in the accompanying figure, drawn from the average findings in the series of experiments.

It has been shown by Baglioni (5), and also by Bethe (6) and by Kuiper (7), that the respiratory rate of fishes varies with the temperature of the surrounding medium. The present experiments confirm this finding. It should be mentioned, however, that even at the same water temperature different fishes show slightly varying respiratory rates.

At some of the lower water temperatures (6° to 10°) the respiratory act is practically coincident with that of the heart-beat. The graph indicates this condition and also a striking divergence of the respiratory from the pulse curve at the higher temperatures. Changes in surrounding temperature are therefore seen to affect the respiratory mechanism more profoundly than the circulatory system.

At the extreme temperatures (−1.9° and 30°) respiration was often irregular, and Cheyne-Stokes breathing was observed on several occasions. Gasping and asphyxial respiration also sometimes supervened, quickly followed by the complete arrest of spontaneous respiratory movements. In this eventuality the gill covers and gill bars in teleosts, and the pseudobranchs in the skate, were maintained in rigid extension.

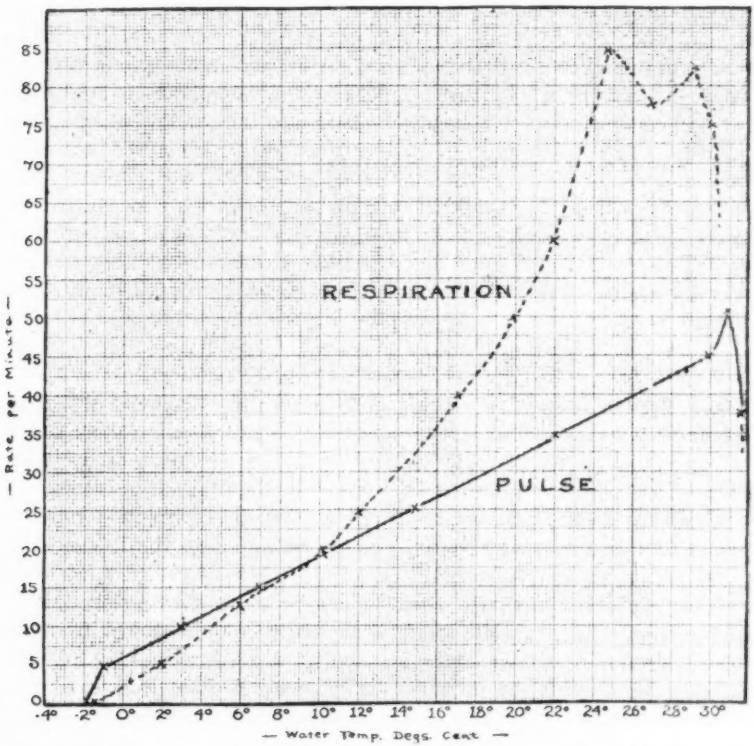


Fig. 1. Respiration and pulse rates in fishes at different surrounding water temperatures.

Attempts to upset this condition by digital pressure over the branchial region only resulted in its immediate reestablishment on withdrawal of the pressure, but a reversion of the surrounding temperature toward the optimum occasionally brought about normal breathing once more.

Reflexes and order of recovery. The following reflexes were observed after the disappearance of all spontaneous movements in the

various species of fishes investigated when subjected to extremes of either high or low temperatures:

Respiratory reflex. Occurs on tapping with a glass rod or (sometimes) on pinching in either the pectoral or the cloacal regions. Single or repeated respirations were evoked, according to the condition of the fish and the strength of the stimulus.

Fin reflexes. Caudal, pelvic and pectoral, and dorsal and ventral fin reflexes, elicited on touching or tapping within areas about the respective fin attachments. Pectoral and pelvic fin responses were usually bilateral on stimulating in or about the mid-line region, and unilateral on stimulating at the fin bases. In two forms, namely, *Hemitripterus americanus* and *Zoarces anguillaris*, a peculiar reflex elevation and homolateral inclination of the dorsal fins occurred on gentle stroking of the skin of the dorsum.

Clasper reflexes. In the skate, homolateral flexion of the clasper was evoked on tapping any part of the organ; on similar stimulation of the edge of the pelvic fin, however, a peculiar reflex was elicited, the contralateral clasper being very sharply and extremely drawn toward the receptor area.

Clasper pouch reflex. Extreme eversion of the clasper pouch of the skate on sudden subjection of the fish to extreme temperatures.

Spine reflex. Elevation of cephalic spines and gill cover of *Myoxocephalus octodecimspinosus* and *Myoxocephalus greenlandicus* on handling and often on merely tapping the fish. This is obviously a protective reaction.

Swallowing reflex. Observed in *Hemitripterus americanus* only, at normal temperatures, and readily evoked on handling the fish either in or out of the water. As a result, large quantities of either water or air are taken into the stomach, and the fish presents the appearance of a chimerean monster. In the event of air being swallowed, the fish flounders helplessly on its back at the surface when re-introduced into water, and soon dies if left in this manner. It is extremely difficult to expel either air or water from the stomach by pressure with both hands, but the condition may be immediately relieved, and recovery of the fish occurs, if a glass tube is passed from the esophagus into the stomach, thus allowing ready escape of the fluid to the exterior. It is worthy of mention that this phagitic habit of *Hemitripterus americanus* is exercised only on taking the fresh fish from its natural surroundings, and does not occur during captivity.

After complete submersion of all visible reactions in fishes by exposure to low temperatures, the following order of recovery on elevation of the surrounding temperature has been again and again observed: *1*, heartbeat; *2*, oral and clasper (skate) reflexes; *3*, caudal and pelvic fin reflexes;

4, pectoral, dorsal and ventral fin reflexes; *5*, respiratory reflex on stimulation; *6*, spontaneous movements of gill covers; *7*, asphyxial respiration; *8*, uncontrolled swimming movements; *9*, rhythmic movements; *10*, vision; *11*, equilibration and normal natation. As far as could be ascertained, a similar order of recovery occurs after the exposure of fishes to high temperatures.

SUMMARY

Many species of fishes survive exposure to water at temperatures from one to two degrees below zero for one to several hours. In this period all vital activities gradually diminish and eventually disappear, the heart-beat being the last to cease, in diastole. A fish in such condition appears stiffly frozen and altogether lifeless, but resuscitation may occur on slowly increasing the temperature of the surrounding water.

The order in which general and specific life processes reappear after suspension by extreme cold is noted in detail.

Elasmobranch fishes show greater resistance to cold than teleosts.

The heart of the skate may be solidly frozen for several hours and yet on thawing recover its regular rhythm.

Exposure to a water temperature of 25° is withstood by fishes with little difficulty, but at 30° much distress is shown and death soon follows unless the water temperature is reduced.

Recovery of a fish may occur after suppression of respiration and possibly of the heart-beat by exposure to high temperatures.

The rate of the heart-beat has been studied in the intact fish and is found to vary directly with the temperature of the surrounding water.

A number of piscine reflexes are described.

My best thanks are due to Prof. A. G. Huntsman, the Director of the St. Andrew's Biological Station, for his generous assistance.

BIBLIOGRAPHY

(1) HUNTER: Works, London, 1837, iv, 131.
(2) REGNARD: Compt. rend. Soc. de biol., 1895, 652.
(3) PICTET: Quoted from PUTTER: Vergleich. Physiol., 1911, 386.
(4) MARTIN: Contrib. to Can. Biol., 1911–14, Fasc. i., 73.
(5) BAGLIONI: Zeitschr. allg. Physiol., 1908, vii, 248.
(6) BETHE: Allgemeine Anatomie und Physiologie des Nervensystem, 1903, 404.
(7) KUIPER: Pflüger's Arch., 1907, cxvii, 1.

A STUDY OF VASECTOMY ON RATS AND GUINEA PIGS[1]

ROBERT OSLUND

From the Department of Zoölogy, University of Chicago

Received for publication September 18, 1923

This investigation began as an attempt to make an analysis of the changes in the testis following vasectomy. Leon Gosselin (1853) introduced the experimental study of effects of ligation and resection of the vas deferens using dogs as experimental material. He found that there was normal spermatogenesis present four to six months after such operation. Brissaud (1880) found the same situation in rabbits. Ancel and Bouin (1903), using rabbits and pigs, secured exactly the opposite results. They stated (p. 499) that the testicles they had experimentally transformed resembled in all respects the testicles of cryptorchid animals, i.e., with seminal tubules reduced and having a single layer of columnar Sertoli cells prolonged into filamentous processes, and with no sperm-forming cells present. Shattock and Seligman (1904), studying sheep, obtained results contrary to those of Ancel and Bouin and stated (p. 52) that "microscopic section of the testis of the normal adult Herdwick sheep and of that from the vasectomized animal of the same age and killed at the same date, show similar histological pictures." Later investigators, although performing the same operation upon rats, guinea pigs and rabbits, have obtained conflicting results. Thus Steinach (1921), Sand (1921) and Lipschutz (1922) have reported degenerative changes followed by regeneration. The last named states that in some animals one testis may undergo degeneration while the other testis remains normal. Sand, on the other hand, finds in many experiments that degeneration does not occur. These are but a few of the workers in this field, but further references would only serve to extend the contradictions already seen. It is evident from these instances that the effects upon the testes of vasectomy operations are not always the same, either because of anatomical and physiological differences in the various animals used, or because of slight variations in the operative technique. In some instances there have also been made inadequate analyses of the situations encountered.

[1] A dissertation submitted to the faculty of the Ogden School of Science in candidacy for the degree of Doctor of Philosophy.

Since there are so many discrepancies in accounts of these operations, it was decided to repeat the experiments on a large scale: constant results were finally obtained and it is the purpose of this paper to give a detailed account of the operative procedures involved, and a report of the findings with analysis of them. It became evident during the early part of this work that the discrepancies in the results obtained were due to a confusion of results of vasectomy and of artificial cryptorchidism. It was found that ligation and resection of the vas deferens does not of itself produce degeneration of the germinal epithelium, and that when such degeneration followed vasectomy it was due to the fact that the testes remained in the abdominal cavity following the operation: such a displacement of itself (artificial cryptorchidism) is sufficient to produce degeneration of the germinal epithelium without vasectomy.

MATERIAL AND TECHNIQUE. Because of the fact that most of the preceding work was done on rats and guinea pigs and because of the availability of these animals they have served as the experimental material of this paper. The rats are of the Wistar Institute strain, of which there is a large, well-kept colony at this laboratory. The guinea pigs are from a local colony of healthy animals. The caretaker knew no distinction in the stock and gave all animals the same care. That the diet was suitable was judged from the health of the colony and from the large size of the litters raised. Controls were from the same groups, often blood brothers, and killed at the same age as the experimental animals.

All operations were performed with aseptic precautions. The animals were etherized, the abdomen clipped of hair, shaved and then washed with an alcoholic solution of iodine. Laparotomy, along the mid-ventral line, was then performed exposing the vasa deferentia, internal spermatic arteries and fat-bodies attached to the testes. In some animals the testes were found in the abdominal cavity, as is common in rodents, while in most cases they were located in the scrotum. At the conclusion of the operation the incision was closed by a continuous suture first of the muscles and then of the skin.

In order to cover the field comprehensively the following modifications of the general procedure were carried out: *1*, unilateral vasectomy; *2*, bilateral vasectomy; *3*, unilateral vasectomy with the contralateral testis removed; *4*, bilateral vasectomy with both testes in the scrotum; *5*, bilateral vasectomy with one testis in the scrotum, the other testis retained in the abdominal cavity; *6*, unilateral vasectomy with the contralateral testis retained in the abdominal cavity; *7*, artificial cryptorchidism; and *8*, partial castration. Rats ranging in age from thirty days to senescent animals and guinea pigs from twelve days to adults of

more than one year have been used; totaling over two hundred animals.[2] (Only a representative portion of the experiments is here given.) The animals were killed after periods of from fourteen days to ten months from the time of the operation. Gross peculiarities were noted at that time and the condition of the vas deferens—whether or not it had regenerated—was determined. During the later part of the work the importance of the location of the testes became known. Special attention was then devoted to controlling the place of their residence and in recording the same.

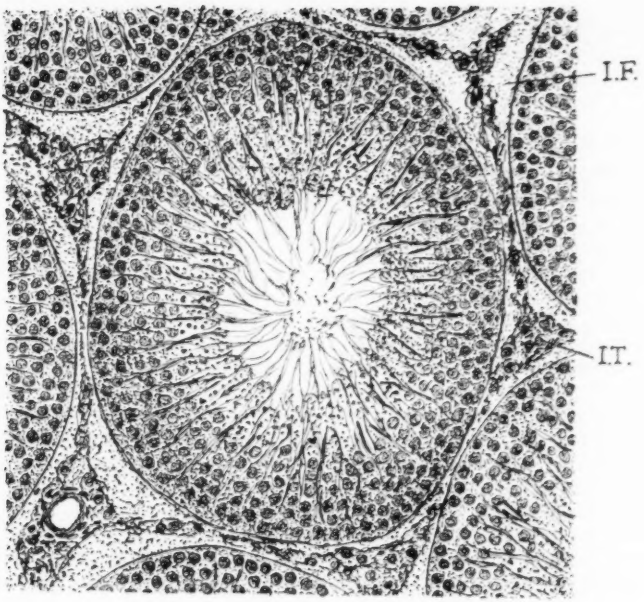
In the preparation of the tissue for study, Allen's (1919 a) injection method of fixation was used. In most cases his modification of Bouin's fluid was employed as the fixing agent, but as the work progressed Bouin's fluid and Formol-Zenker were tried. No important variations were noted in the results from these fixatives that might be attributed to them. The tissues were dehydrated and embedded in paraffin in the manner described by Allen (1919 a) and sections were cut 7 microns in thickness. Many combinations of stains were tried and from each experiment a set of slides stained with Mallory's triple, Harris' hematoxylin and eosin and Delafield's hematoxylin and congo-red were prepared. All comparative observations were made upon representative sections taken from corresponding regions of the different testes.

Although microscopic observations were most relied upon, the condition of the testis could in most cases be recognized by the naked eye, both before and after sectioning. In cases of degeneration the testis was smaller, the tubules contracted and almost devoid of the content. The contrast in microscopic section is plainly seen in figures 1 and 2 drawn to the same scale and of the same animal.

EXPERIMENTAL DATA. For convenience of presentation the experiments have been divided into two groups which, in general, indicate the progress of the investigation. Each group has been subdivided according to the type of operation performed and the results obtained. The experiments of the first group presented were performed before the importance of the position of the testis was suspected. In these experiments, therefore, the location of the testis was seldom noted. The second group comprises those experiments performed during the later part of the work when the variable factor (location of the testis) was controlled.

Group I. Location of the testis not known. A. Degeneration of the germinal epithelium was found to follow unilateral vasectomy in some

[2] This study was taken up at the suggestion of Prof. F. R. Lillie and is the outgrowth of a series of experiments done at the University of Chicago by Dr. C. R. Moore. I wish to acknowledge my indebtedness to them for interesting me in this problem.



All figures are from camera lucida drawings by Jose K. Santos

Fig. 1. Portion of right testis from rat 38 no. 36. *IF*, intertubular fluid. *IT*, intertubular tissue—interstitial and connective tissue.

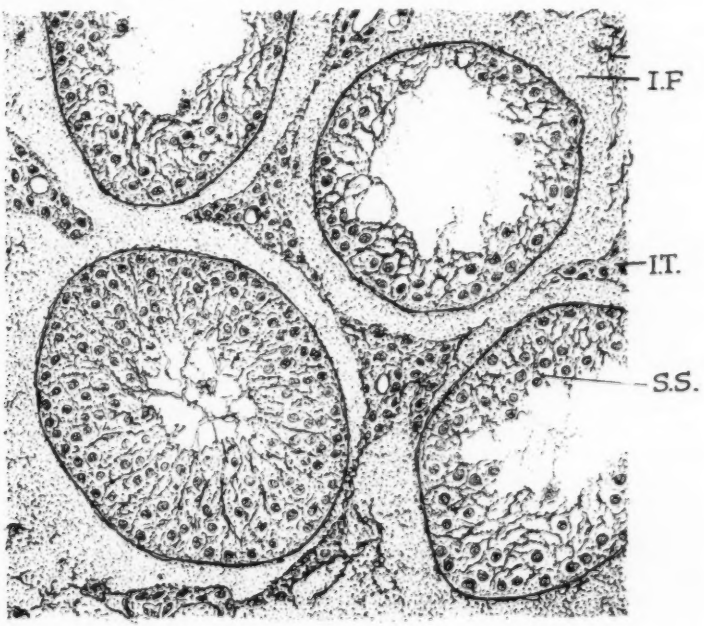


Fig. 2. Portion of left testis from rat 38 no. 36. *IF*, intertubular fluid; *IT*, intertubular tissue; *SS*, syncytium of supporting epithelial cells.

of the experiments, while in others no changes could be found. Examples of each type of results will be given.

Protocols of two cases where degeneration was found to follow unilateral vasectomy are here given:

Guinea pig 40 no. 1. Age about one year. The left vas deferens was ligated in two places with a coarse silk thread and resected between the ligatures. The testis of which the vas deferens had been ligated was then allowed to return of its own accord to the scrotum while the incision was closed. Six months later the animal was killed and at that time the vas deferens was closed by the ligature. The microscopic picture of the left (operated) testis showed that the germinal epithelium had undergone considerable degeneration. Many of the seminiferous tubules were entirely devoid of spermatogenic cells, many had very few active cells while the remaining tubules were full of debris. There was no modification from the average normal condition found in the inter-tubular tissue, either in quantity or in appearance of the cells. The right (unoperated) testis was normal in all respects.

Rat 24 no. 2. Operated upon when three months, eleven days of age. The left vas deferens was ligated and resected, a piece about one-fourth inch in length being cut out. Two months later when the animal was killed the vas deferens was found to have grown together and the lumen of the same was complete. Microscopic sections showed that many of the seminiferous tubules were almost devoid of active cells, while adjoining tubules were full and appeared entirely normal.

Were these, and a few others that might be selected, the total number of experiments performed, there would be little reason for doubting that degeneration of the germinal tissue results from ligation of the vas deferens. Such results are, however, not found in the immense majority of the experiments.

In another selected group of experiments involving unilateral vasectomy no changes were found following the operation. Protocols of four such animals follow:

Guinea pig 25 no. 5. Age, four months. The right vas deferens was resected only, the testicular end opening into the peritoneal cavity, the urethral end being ligatured. When the animal was killed eight months later the testicular end of the vas deferens had grown closed. Sections of the right (operated) testis, as those of the left testis, showed normal tissue throughout.

Guinea pig 5 no. 27. Age, twelve days. The left vas deferens was ligated and resected. The ligature was still firm when the animal was killed eight months later. Microscopic sections of both testes were alike and normal in every respect.

Guinea pig 11 no. 9. Age, thirty days. The right vas deferens was ligated and resected. When the animal was killed ten months later the vas deferens appeared to have regenerated. The microscopic sections of both testes were normal.

Rat 23 no. 1. Age, ninety-four days. The left vas deferens was resected, the urethral end only being tied. When the animal was killed one month later a cyst had formed over the end of the resected vas deferens. This cyst was about the size of a normal rat testis, and filled the mouth of the inguinal canal. The testis was below the cyst in the scrotum. Both testes were normal. It might be argued that the cyst formed an escape for the products of the seminiferous tubules and that the testis should

not, therefore, be looked upon as having the vas deferens closed. But many cases were found where most of the germinal epithelium had degenerated in the testis, in spite of the presence of a cyst on the vas deferens; for example, in rat 51 no. 23 (see below). Again it might be said that two months was not time enough for the degeneration to have taken place. However, in rat 24 no. 2 given above, many of the seminiferous tubules were almost devoid of active cells one month after the operation.

In none of these cases has there resulted any change in the tissues following ligation of the vas deferens. Yet the procedure was supposedly the same as in those cases given above where degenerative changes were found. In none of these experiments was there any effort made to determine the location of the testis during the period following the operation, until the animal was killed. The inguinal canal was open and the gubernaculum remained attached and it was therefore assumed that the testes would take their normal position when the wound was closed.

B. When the experiments involving bilateral vasectomy were checked over, three classes of results were found, viz: 1, those in which both testes showed degenerative changes; 2, those in which neither testis showed any degenerative changes; and 3, those in which degenerative changes were found in one testis, the other testis being normal. Each of these groups will be taken up separately.

Protocols of animals in which degeneration took place in both testes following bilateral vasectomy will be given first:

Guinea pig 31 no. 36. Age, four months. Both vasa deferentia were ligated and resected. The animal was killed one month later at which time both ligatures on the vasa deferentia were still firm. Microscopic sections showed that degeneration had taken place in most of the tubules of both testes. This degeneration was more evident in the right testis than in the left where many of the tubules were normal.

Rat 51 no. 23. Over one and one-half years of age. The testes appeared normal at the time both vasa deferentia were ligated and resected. The rat was killed four months later at which time a large cyst was found to have grown over the end of each vas deferens and adhesions were holding the entire testicular mass of each side in the abdominal cavity. Sections of each testis showed many seminiferous tubules entirely devoid of spermatogenetic cells, while other tubules appeared normal and full of active cells. This experiment was referred to in a former group as one in which degenerative changes were found to have taken place though a large cyst (in this case each cyst was larger than a normal rat testis) closed the end of the vasa deferentia.

In this group where both vasa deferentia were ligated, there followed degenerative changes, though in one case one testis had undergone more degeneration than the other testis of the same animal. These results are, in general, in agreement with statement of some previous workers, though they are not uniform and therefore somewhat confusing.

In another group of experiments involving bilateral vasectomy the results were of the opposite nature; no degeneration took place. The operations were performed in the same manner as in those experiments

just cited and at the same time. Protocols of four such cases will be given:

Guinea pig 37 no. 6. Age, eight months. Both vasa deferentia were ligated and resected. The animal was killed six months later at which time both ligatures were found to be firm. Microscopic sections of each testis were those of normal testes. No degeneration was found.

Guinea pig 29 no. 34. Age, four months. Both vasa deferentia were ligated and resected. When the animal was killed six months later both vasa deferentia were still closed by the ligatures. Microscopic observations indicated that both testes were normal and free from degenerative changes.

Guinea pig 21 no. 26. Age, two months. Both vasa deferentia were ligated and resected. When the animal was killed two months later, the ligatures were still firm. No changes were found to have taken place in either testis. In none of these three cases were there any cysts present complicating the findings. In guinea pigs 37 no. 6 and 29 no. 34, though the vasa deferentia were ligated during a period of six months, no degeneration followed.

Rat 35 no. 31. Adult. Both vasa deferentia were ligated and resected. When the animal was killed six months later the left vas deferens was closed, but the right vas deferens may have regenerated. This could not be ascertained. Microscopic sections showed that both testes were normal.

In still another group of experiments involving bilateral vasectomy another type of result was found. One testis underwent degeneration while the other testis remained normal. Examples of such experiments follow:

Guinea pig 38 no. 7. Age, eight months. Both vasa deferentia were ligated and resected for a period of six months. Microscopic sections showed that the left testis only had undergone degeneration; the right testis was normal.

Guinea pig 2 no. 12. Age, 12 days. Ligation of both vasa deferentia was complete for a period of ten months. Microscopic observation showed that the germinal epithelium of the right testis only had undergone degeneration; the left testis was normal.

Guinea pig 13 no. 19. Age, thirty days. Though the vasa deferentia were ligated for a period of ten months, the left testis did not undergo degenerative changes. There was some degeneration found in the right testis.

Guinea pig 27 no. 32. Age, four months. Both vasa deferentia were ligated for a period of six months. Degenerative changes were found in the tubules of the right testis while the left testis was normal.

Rat 36 no. 34. Adult. Both vasa deferentia were ligated for a period of six months. When the animal was killed a small adhesion was holding the right testis at the mouth of the inguinal canal. Degenerative changes were found in the right testis only.

In these five experiments the ligation had been for periods of six and ten months. In each case degeneration took place in one testis only, while the other testis remained unaffected by the operation. Since the differences here seen are within the individual animals (the testis of one side as opposed to that of the other side), it cannot be said to be due to the physiological condition of the animals.

By way of summarizing the results of the five groups of experiments we find:

1. Unilateral vasectomy, followed by degeneration of the germinal epithelium.

2. Unilateral vasectomy, followed by no degenerative changes.

3. Bilateral vasectomy, followed by degeneration of germinal epithelium in both testes.

4. Bilateral vasectomy, followed by no degenerative changes in either testis.

5. Bilateral vasectomy, followed by degeneration of the germinal epithelium in one testis only, the other testis remaining normal.

These experiments were all performed as nearly alike as was possible and the dates of operation overlap so that no improvement in technique entered into account. The results were inconstant, and especially in the last group there is a marked irregularity. Time cannot be a causal factor in these experiments since degeneration in one case (guinea pig 31 no. 36) occurred in one month, and in other cases did not occur in ten months.

Group II. Location of the testis known. It is obvious that factors not yet accounted for cause or prevent the degenerative process. It was with the hope of isolating each influence, that many more operations were performed and the technique carefully checked. A clew to the situation had been found in experiments guinea pig 24 no. 4 and in rats 51 no. 23 and 36 no. 34 previously cited, where it was noted that in each case the testis that had undergone degeneration was retained in the abdominal cavity by an adhesion. As has been stated, no attention was devoted in the preceding experiments to determining the location or the condition of the testis between the date of operation and that of death. No definite reference to the location of the testis after vasectomy operations occurs in the literature as far as the writer is aware. Accordingly, a series of operations involving bilateral vasectomy was performed, using the same technique as before, but observing, during the post-operative period, the location of the testes. In some of the experiments of this series the testes were in the scrotum following ligation of the vasa deferentia while in other experiments they were retained in the body cavity. Each type of operation will be considered separately.

A. In one group of animals both testes were known to reside in the scrotum following the vasectomy operation until the animals were killed. Protocols of such cases follow:

Guinea pig 20 no. 25. Adult. Both vasa deferentia were ligated and resected. When the animal was killed forty-five days later the ligatures on the vasa deferentia were firm. The testes were in the scrotum and their location had been ascertained throughout the post-operative period by palpation. Microscopic observations showed that no degeneration had taken place in either testis.

Guinea pig 21 no. 26. Age, two months. Both vasa deferentia were ligated and resected. During the post-operative period of two months, the location of the testes was ascertained to be in the scrotum by palpation from time to time. Neither testis showed any degenerative changes.

Guinea pig 23 no. 54. Age, two months. Both vasa deferentia were ligated and resected. During the three months following it was ascertained, by palpation, that the testes were in the scrotum. Both testes were found to be normal in all respects.

Rat 39 no. 37. Adult. The vasa deferentia were ligated and resected. Throughout the post-operative period of five months, the testes were known to be in the scrotum. There were no signs of degeneration in the prepared slides of either testis.

Rat 16 no. 42. Age, forty days when bilateral vasectomy was performed. The animal was killed four months later, and during this time the testes were known to be in the scrotum. Microscopically, both testes were normal.

Rat 42 no. 55. Adult. Ligation of the vasa deferentia was for one month. The testes were in the scrotum during this period, and both were normal when the animal was killed.

In this group of six animals, the testes were known to be in the scrotum from the date of operation until killed. In rats one can usually determine the presence of the testes in the scrotum by observation, but in cases of doubt these animals were palpated. In guinea pigs palpation was always resorted to in order that there might be no uncertainty. In none of these cases was there found any indication of changes in the testes from the operation. The microscopic picture of each testis was normal.

B. In order to determine the full significance of these findings another series of operations was tried. In this series the testes were known to be in the scrotum following the operation. Thirty to sixty days later one testis was pulled from the scrotum into the abdominal cavity and there made fast to the belly wall. Four such experiments are here given:

Rat 43 no. 56. Adult. The vasa deferentia were ligated and resected. The testes were ascertained, by palpation, to be in the scrotum during a period of thirty days following the operation. A second operation was then performed in which the right testis, then normal in size, was pulled from the scrotum into the abdominal cavity and lightly stitched to the abdominal wall by passing a silk thread through the fat-body of the testis and through the peritoneum and an abdominal muscle. During the fifteen days following, the left testis remained in the scrotum, the right testis in the abdominal cavity. The animal was then killed; both vasa deferentia were closed by the ligatures and the testes were noted to be in the locations desired in the experiment. It was noted that the right testis was not more than one-half its former size as seen at the time it was fastened in the body cavity or of that of the left testis. When microscopic examinations were made it was found that the left testis was normal and full spermatogenesis was in progress. In the right testis, on the other hand, the germinal epithelium had almost completely degenerated. This had happened presumably in fifteen days.

Rat 45 no. 58. Adult. This animal was operated upon in the same manner as the preceding with the exception that the left testis, then normal in size was fastened to the abdominal wall at the end of the first thirty days instead of the right testis. When the animal was killed fifteen days later (forty-five days after the initial operation) the

ligatures on both vasa deferentia were firm. The left testis was no more than one-half its former size as noted at the time it was fastened to the abdominal wall or of the right testis. Microscopic observations revealed the fact that the right testis was normal, while the seminiferous tubules of the left testis were almost devoid of spermatogenic content.

Rats 44 no. 57 and 46 no. 59, both adults, were operated upon in the same manner as those just described. In these two cases, however, thirty days elapsed between the date of the second operation and that of killing. The results were similar, except that degeneration was here more complete in the testes retained in the abdominal cavity. The testes that remained in the scrotum following vasectomy were normal.

C. The results of the above four experiments seemed conclusive, but in order that there might be no uncertainty in the matter another series of experiments was prepared.

Four rats were used: 29 no. 32, 30 no. 39, 31 no. 49 and 28 no. 52, the first three were four and one-half months old, the last four months old. Both vasa deferentia were ligated and resected in each animal; for a period of three months after the operation the testes were ascertained by palpation to be in the scrotal sacs. In a second operation made three months after the first, the left testes, then normal in size, were pulled into the abdominal cavity and there made fast to the belly wall as in former experiments. These animals were killed fifteen days later, three and one-half months after the initial operation. The ligatures were all firm and in each case the left testis was about one-half its former size as noted at the time of the second operation or of that of the right testis. In every experiment the right testis was normal in microscopical structure while the left testis was almost devoid of its germinal content. This condition is shown very well by the illustrations, figures 3 and 4, showing representative portions of the right and the left testis of rat 30 no. 39 of this series. From these experiments it is very evident that ligation of the vas deferens does not of itself cause degeneration, but when the testis, after ligation of the vas deferens, is retained in the abdominal cavity, degeneration always follows very rapidly.

In order to further determine the relative importance of these two factors (ligation of the vas deferens and retention of the testis in the abdominal cavity) as causes of the changes produced in the testis a crucial experiment was devised.

Rat 38 no. 36. Adult. After the laparotomy the right vas deferens was ligated and resected without removing the testis from the scrotum. The left testis was then carefully pulled from the scrotum and fastened in the abdominal cavity without in any way injuring the vas deferens of that side. As in former experiments the thread fastening the testis to the belly wall passed through only fatty and connective tissues of the testis and in no way injured the tunica albuginea or the content of the testis. The rat was killed one month later at which time the ligature on the right vas deferens was still firm and the left testis, now no more than one-half the size of the right testis, was retained in the abdominal cavity. When microscopic preparations were examined

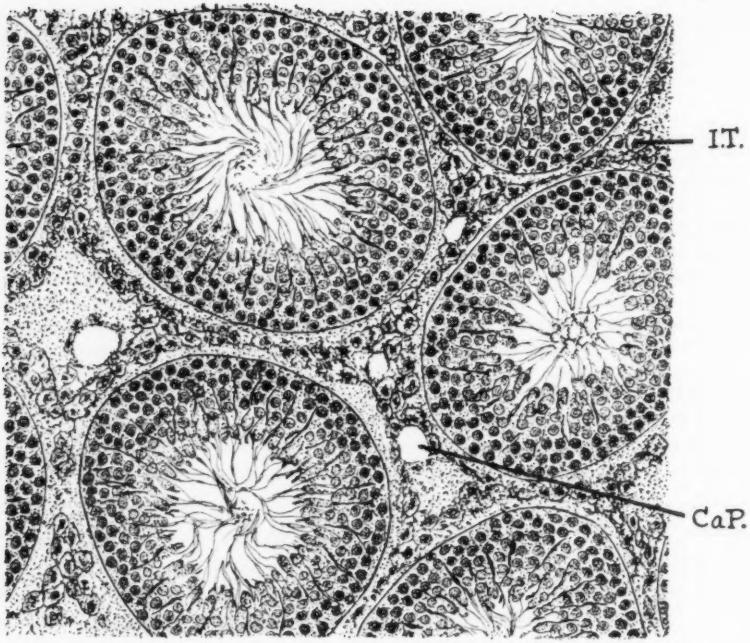


Fig. 3. Portion of right testis of rat 30 no. 39. *IT*, intertubular tissue; *Cap*, capillary.

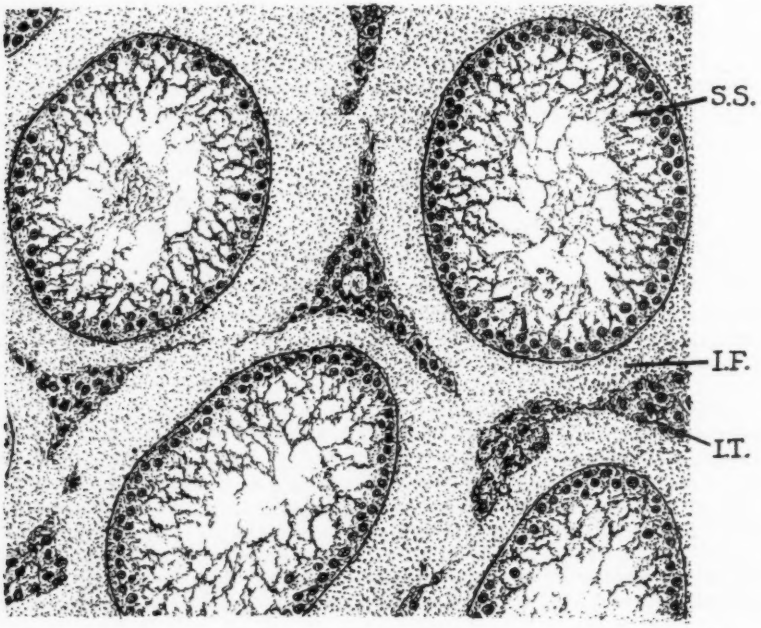


Fig. 4. Portion of left testis of rat 30 no. 39. *IF*, intertubular fluid; *IT*, intertubular tissue; *SS*, syncytium of supporting epithelial cells.

it was found that the right testis (with ligated vas deferens) was normal, while the left testis which had been retained in the abdominal cavity but with vas deferens uninjured, had undergone extensive degeneration. The seminiferous tubules were almost empty and there was some hypertrophy of the intertubular tissues. Figures 1 and 2 represent typical tubules of the right and left testes of this animal, and show in a striking manner the relative importance of retention of the testes in the abdominal cavity in contrast with ligation of the vas deferens.

Rat 54 no. 28. Adult. Both testes, without injury to the vasa deferentia, were fastened within the abdominal cavity. Four months later when the animal was killed it was found that the left testis had become free and returned to the scrotum. Microscopic examination showed that both testes had undergone degeneration, but that the left testis was more nearly normal than the right testis. It was inferred that the testis upon returning to the scrotum had partially recovered from the degenerative changes undergone while retained in the abdominal cavity. The interstitial tissue between the tubules seemed packed more closely among those tubules that contained cells undergoing active spermatogenesis. Between neighboring tubules, where spermatogonia were abundant but where only a few spermatocytes were seen, the interstitial tissue appeared more loose and more nearly like that of the right testis. Division figures in the spermatogenic cells appeared more abundant than in the normal testes and the inference that regeneration took place in this testis upon returning to the scrotum therefore seems justified.

This experiment shows that retention of the testis in the abdominal cavity produces degeneration of the germinal epithelium, and that this (artificial cryptorchidism) has been an influence in the above experiments where degeneration has been produced. Sand (1921) has described similar experiments where one vas deferens only was ligated and the contralateral testis, with vas deferens intact, was fastened in the abdominal cavity. On page 522, table III, he gives the results of three experiments of this nature. Sand was interested in the interstitial cell phenomena and made no comment upon the material from the germinal tissue viewpoint, other than to record the general findings. An analysis of his experiments and their results indicates that ligation of the vas deferens (in these cases, nine months, nine months nine days, and five months respectively) has not caused atrophy of the tubular content. Retention of the testis (vas deferens not ligated) has in each case caused atrophy of the tubular content and hypertrophy of the interstitial tissue.

Partial castration. In view of the fact that very little hypertrophy of the interstitial tissue resulted in my experiments a series designated as "partial-castration experiments" was tried. In these experiments only a small portion of one testis was left in each animal. The technique used was the same as that employed by Lipschütz (1922). This series consists of eleven experiments, ten of which agree in general with the results described by the above worker, i.e., in complete degeneration, resorption or in cyst-formation of the remaining piece. My experiments do not, however, admit of as simple an explanation as Lipschütz gives for his series. Certainly the results cannot be interpreted in terms

of blood supply alone as Lipschütz supposes. Guinea pig 47 no. 41 of this series lived three months after the operation. When killed, the fragment left in the animal was found in the scrotum and, when sectioned, the tubules were found to be full of spermatogenetic tissue and to all appearances normal. In the remaining ten cases the testis fragments were found in the abdominal cavity and devoid of germinal epithelium. Though there are several factors present complicating the results of these experiments, the fact that germinal epithelium was present in the one case where the fragment was located in the scrotum supports the thesis that location of the testis is here a prime factor as was found in vasectomy experiments.

Throughout these experiments the interstitial cell phenomena have been studied, but the influencing conditions have been so numerous that a solution of this phase of the problem has not been attained. Very little hypertrophy of interstitial tissue and certainly no compensatory hypertrophy was found. A full discussion of the influencing factors and relationships found in these experiments has been reserved for a later paper.

Discussion. It seems conclusively proved that degeneration of the germinal epithelium results from displacement of the testis from the scrotum to the abdominal cavity and that vasectomy alone does not cause degenerative changes in the testis. In every experiment in which the testis was retained in the abdominal cavity, the spermatogenetic tissue underwent degeneration. In some of the experiments the vas deferens had been ligated before the testis was fastened in the abdominal cavity, but in the last two experiments, given above, and in the series by Sand, above cited, the testes were placed in the abdominal cavity without injury to the vas deferens. Degeneration had in each case resulted from displacement of the testis. On the other hand, degeneration of the germinal epithelium has not followed ligation of the vas deferens in any experiment where the testes were known to reside in the scrotum following the operation. In many of the experiments given above and in many of those not here included, ligation of the vas deferens had been for a period of ten months without any degenerative effect upon the testes. The size of the testis, the size of the attached fat-body and the size and shape of the inguinal canal, are all factors here involved.

The above explanation satisfies all the conditions met with in these experiments and all thus far met with in the literature where sufficient data are given to make an interpretation possible. In the postulate that degeneration of the germinal epithelium is a function of the location of the testis, the conditions met with in all cases of vasectomy are satisfied.

Other views have been presented by writers to explain the variable results. Ancel and Bouin have stated that degeneration always resulted

if the experiment was continued long enough, but in many of my experiments no change took place in the testis even after ligation and resection of the vas deferens for a period of six to ten months. It would seem that at least one-third the span of the animal's life would be ample time for the appearance of the changes, especially since degeneration has been found in many cases within fifteen days, when the testes were fastened in the abdominal cavity.

Sand (1921), while calling attention to the irregularities in his experiments, explains them by saying that while degeneration takes place quickly in adults, it does not commence until after puberty. On page 499 (1922) he presents a table showing the results of operations on two rats and two guinea pigs. In his summary he states that in four cases of bilateral vasectomy performed on young animals no distinct effect was produced after five to twelve months and only in one case (M. 114) was there any effect and in that experiment beginning after the twelfth month. It is the youngest animal in his series that shows the degeneration and it is probable that the testes in the other case were normal because they returned to the scrotum. On page 520 he gives another table showing that the testis of a rat twenty-one days of age (C. 154) when retained in the abdominal cavity underwent degeneration. In his series, cited to show that degeneration does not take place in young animals, a number of cases three months of age are included. Lipschütz (1922) on page 133, says, "Sand showed that the remaining testicle can undergo the same hypertrophy even when the vas deferens is ligatured; this is due to the fact that, even after the vas deferens is ligatured, the spermatogenesis can proceed in a normal way, and the degeneration of the tubules begins only when the spermatogenesis is more or less completed."

The above authors do not state their criteria for "young animals" but the writer finds that spermatogenesis is in progress and spermatozoa present in rats and guinea pigs before they are three months of age and extensive degeneration was found following vasectomy in a rat (15 no. 41) only forty days old.

Tiedje (1921) found that when one testis of young guinea pigs is removed and vasectomy performed on the other side, the remaining testis develops normally, but that when such operations are performed on older animals, there is at first degeneration followed by complete regeneration. He performed the operations by laparotomy and the writer's experience indicates that in young animals the testes readily return to their normal position in the scrotum, while in adult animals, due to factors to which attention had already been called, they often remain in the abdominal cavity after such operations.

Of eleven experiments by Tiedje, eight of the animals showed, according to his interpretation regeneration after degeneration, while three

remained in the degenerated condition. He offers no data to prove his assumption that degeneration actually took place, and was then followed by regeneration. In the eight experiments, Tiedje ligated and resected the vas deferens distal to the epididymis. In these there was least likelihood of retention of the testes in the abdominal cavity, which accounts for their non-degenerated condition. In the other three cases he ligated and resected between the epididymis and the testis which involves a greater amount of handling of parts with a greater probability of trauma and adhesions within the abdominal cavity. This would account for their degenerated condition.

The assumption of degeneration with subsequent regeneration is also made by Steinach (1921) in his experiments on rats and guinea pigs. Like Tiedje, however, he gives no data to prove that this is the usual course of events, but the conclusion is derived from comparisons of cases where degeneration was found in the testes of animals killed shortly after vasectomy with others in which no degeneration was found in testes of animals that had lived for a longer time following vasectomy. In his operations he not only pulled the testes into the abdominal cavity, but while examining them he pulled them out into the open. He then ligated the vasa deferentia and returned the testes to "their former site." The more the testes are handled, the more likely is it that trauma will take place and adhesions form, which would account for the cases of degeneration. But it is also a common occurrence for testes to return to the scrotum and thus the cases in which there is no degeneration are readily explained.

Myers (1915) reported on a series of rats subjected to vasectomy by Stotsenberg in which he describes degeneration of increasing completeness the longer the animal had lived following the operation. No regeneration was found in that series.

If occlusion of the duct is sufficient to cause degeneration it does not seem reasonable to assume that regeneration can take place until the causal factor has been removed, even if the experimental analyses were ignored. The writer has been unable to arrange his experiments in any order that would bear out the contentions of Steinach or Tiedje that degeneration followed by regeneration results from vasectomy. Degeneration is present in experiments of ten months' duration as well as in those of fifteen days.

In seeking an explanation other than that of juvenile state to account for those cases where degeneration did not follow ligation of the vas deferens, Sand (1921) noted that cysts or pocket-like structures often formed at the resected end of the vas deferens. On page 503 he says that he is inclined toward the opinion of Tournade, that the cysts have an importance in the results following resection, but he does not un-

equivocally align himself with that viewpoint. As Sand states, these cysts are not constantly found and their importance in explaining the absence of degeneration is for this reason doubtful. Moreover, in my series of experiments, these cysts have been found fifteen days after operation in cases in which degeneration of the germinal epithelium has nevertheless occurred. In this connection it seems worthy of mention that in many experiments of both short and long duration the tubules of the epididymis were large and distended. Sections of such tubules showed them to be full of normal and degenerating spermatozoa. Sections of the cysts showed them to be full of clumps or nodules of what appeared to be agglutinated spermatogenic cells, but there is no correlation between cyst formation and the condition of the germinal epithelium.

Discussion and comparisons have thus far been limited to rodents. In these animals, at least all those used in this type of experiment, the inguinal canal is open and the testes are free to pass in and out of the abdominal cavity. It is further seen that in all cases when the testes are retained in the abdominal cavity for even a short period (fifteen days) degeneration takes place.

A few workers have used carnivora and with the exception of Kuntz (1921) they report that no changes are found following vasectomy. Shattock and Seligman (1904), working on sheep, state on page 51 that "when the vas is cleanly isolated and divided after ligation without the inclusion of vessels, or without the subsequent occurrence of thrombosis, no atrophy of the gland ensues." The same condition has been found in man, according to all reliable reports, where the vas deferens has been occluded by accident or by disease. In these three orders of mammals the testes are contained in a pendulous scrotum with the inguinal canal closed, and the location of the testes is known to be in the scrotum. The results are therefore not confused by artificial cryptorchidism as when rodents are used. Cooper (1827), using dogs; Curling (1843), using cats and dogs; Gosselin (1853), using dogs; Guyon (1895), using dogs; Wheelon (1921), using dogs; and Wallace (1904), using dogs and cats, have all found that degeneration does not follow ligation of the vas deferens. From a few months to four years elapsed between the date of operation and the date when the animals were killed. In the one series of experiments on carnivora where degeneration was reported as a result of vasectomy the author, Kuntz (1921, p. 198) says of his controls: "One unoperated dog, kept for one month with some of the operated dogs as a control animal, showed degenerative changes in both testes. Another dog was subjected, under ether anesthesia, to an operation not involving the genital organs and kept under essentially the same conditions as the other experimental animals for four months following the operation, when both testes were removed and prepared for study. Preparations of both testes in this

case showed advanced degenerative changes in the seminal epithelium; however, spermatogenesis was not entirely suspended." When the control and experimental animals show like abnormal conditions, no changes can be ascribed as resulting from operative procedures.

The medical literature contains many cases that leave no doubt that no changes occur in the human testis from closure of the vas deferens. A few examples will suffice. Spangaro (1904) found spermatozoa in the testes of two men on whom vasectomy had been performed six months and two and one-half years respectively. Simmonds (1921) examined forty human cases in which one or both vasa deferentia had been closed by cicatrix formation. In thirty cases spermatogenesis was normal. Case histories are not known for the ten cases not accounted for and complications may have been present. Some years ago vasectomy was practised as a substitute for castration for the relief of enlarged prostate gland. It soon became evident that spermatogenesis was not hindered and the relief was not forthcoming while the testis was present. The literature is too voluminous for review here but the great number of such cases reported in that field offers strong evidence that degenerative changes do not result from vasectomy in man. It is therefore evident that in those animals where the testes are suspended in a pedunculate scrotum, where the results are not confused with artificial cryptorchidism, the testes are unaffected by vasectomy. The ligation periods have been from a few months to years and are reported by so many workers that the evidence is unquestionable.

In striking contrast to the results obtained from this type of operation upon these animals, are the results found in cases of cryptorchidism. Among the early workers reporting on such material were Curling (1843), Cooper (1827), Goubaux and Follin (1855), Godard (1856), Monod and Arthaud (1887) and Griffiths (1893). They all agree that cryptorchid testes are devoid of spermatozoa. The material used by these workers varied widely—pigs, horses, dogs and man being included. During recent years many workers have affirmed these findings, among them: Hanes (1911), reporting on pigs, sheep and man; Loeb (1918), on guinea pigs; Goddard (1920), on man, and Moore (1923) on rats. Goddard states (p. 175): "In all the retained organs examined, there was complete atrophy of the contents of the seminiferous tubules." Moore's descriptions of cryptorchid testes fit conditions illustrated in figures 2 and 4 here given. Ancel and Bouin (1903) describing the appearance of the degenerative conditions found by them in testes following vasectomy called attention to the fact that the results resembled in all respects those found in cryptorchid testes. There is every reason to believe that the resemblance was more complete than they supposed. The results they were describing were doubtless those of artificial cryptorchidism and not of vasectomy.

It is the writer's belief that these findings will aid in the interpretation of experiments involving transplantation of ovaries and testes. Ovaries, when used as grafts, generally grow and produce normal germ cells. This fact has made possible the studies by Guthrie (1909, 1911), and by Castle and Phillips (1911), in which the effect of the environment upon the ovum and embryo in guinea pigs was studied. Moore (1921) showed that ovaries transplanted into male animals will grow and produce germ cells for at least eight and one-half months. Grafts of testes, on the other hand, do not produce spermatozoa in the majority of experiments. Though the grafts persist, the seminiferous tubules are empty or contain only spermatogonia. The grafts have usually been placed intraperitoneally, intramuscularly or subcutaneously. If normal, uninjured testes degenerate when placed in the abdominal cavity, one cannot reasonably expect portions of testes to grow in this environment. Those grafts placed subcutaneously should be the most likely to produce normal spermatogenetic cells, since this position is most nearly like that of the scrotum which is a pouch composed of skin and the dartos tunic, and it is these grafts that are most frequently figured in the various papers dealing with testes grafts. That the site of such implantations is important has been shown by Moore (1923), who has found spermatozoa in grafts of rat testes placed in the scrotum.

In birds the anatomical and physiological conditions are altogether different from those in mammals and yet are easily understood from the point of view of location of the testis. Shattock and Seligman (1904) and Massaglia (1920) have shown that ligation of the vas deferens in cocks does not cause degeneration of the germinal epithelium. The former writers found this in all cases while the latter is not so definite and states that the results are not always the same. In birds not only do grafts of ovaries grow, but pieces of testis grafted on the peritoneum grow readily and produce normal appearing spermatozoa. This has been shown by Shattuck and Seligman (1904), Guthrie (1910), Massaglia (1920) and Goodale (1916). The natural environment of both the ovary and the testis in birds is in the abdominal cavity, and when vascularization is not hindered both vasectomized and grafted testes grow and produce spermatozoa.

In brief, it has been shown by various workers that grafts of ovaries of birds and mammals grow readily and produce functional germ cells; that testis grafts in birds likewise produce normal appearing germ cells while testis grafts in mammals do not produce spermatozoa when placed within the abdominal cavity, but do so when they are placed within the scrotum (Moore, 1923). It has also been shown that vasectomy is not followed by degenerative changes in the testis when performed upon birds or upon mammals where the testes reside in a pendulous scrotum. It

has also been shown that in the hands of many experimenters it often happens that no changes are found to follow vasectomy performed upon rats, guinea pigs, and rabbits—Gosselin (1853), Brissaud (1880), Sand (1921) and Lipschütz (1922). Since former workers have failed to note the location of the testes in the ligation experiments and since, in these new experiments, the location of the testes has been controlled, there can be no doubt that the explanation offered is the correct one.

It is well known that the germinal epithelium of the testis is very sensitive to other factors than here described. Paul (1906) found that in rats fed on an exclusive meat diet the reproductive organs were much less developed than in controls. Houssay (1907) and Pezard have conducted similar experiments upon birds, each finding effects leading to progressive sterilization and, according to the former, to loss of sexual dimorphism. It is interesting to note that fattening of the male domestic goose modifies the secondary sexual characters and interferes with the onset of the breeding period (Steive, 1921). Acute alcoholism produces similar results. Bertholet (1909), Wiechelbaum and Kyrle (1912), MacDowell and Vicari (1917), Allen (1919 b), and Kostitch (1921) have all reported degenerative effects upon the germinal epithelium from the use of alcohol. Kostitch examined the testes of many men who died of acute alcoholism and stated (p. 675) that in an advanced state, the tubules appear only as a syncytium, without the layers of spermatogenic cells and consist of no more than a single row of sertoli nuclei. Cordes (1898) reported an aspermatic condition in twenty autopsies of men between the ages of twenty-one and sixty who had suffered but a short time from pneumonia. Wollback (1919) has published similar findings in cases where death was due to influenza. Jacobson (1898) noted that an increase of interstitial cells was associated with inflammation and this is likewise known to be true in orchitis from mumps. In addition to the above some work has been done that indicates that feeding of thyroid interferes with the normal development of the sex organs and the secondary sexual characters (Gudernatsch, 1915; Horning and Torrey, 1922). Meat diet, obesity, alcoholism, fever and inflammation, and possibly thyroid feeding, all produce changes in the testes that closely resemble those found in cryptorchidism.

The evident factor common to all the above conditions is a rise in temperature which is either local or general. In the case of orchitis, there is a local inflammation with accompanying rise in temperature. In obesity, the accumulation of fat in the cutaneous part of the scrotum is sufficient to cause a rise in temperature within. In meat diet, alcoholism, fever and thyroid feeding there is a general rise in temperature of the body which affects the testis, whether its normal residence is in the body cavity, as in birds, or in a scrotum. Where the rise in temperature is general

throughout the body it should affect the ovary as well as the testis and this has been found to be the cause, experimentally, in thyroid feeding and in acute alcoholism. The temperature within the abdominal cavity is appreciably higher than that within the scrotum and it is probable that this is a factor causing degeneration of the germinal epithelium. That this difference of temperature exists has been indicated by the work of Benedict and Slack (1902), showing a temperature gradient of about 5°C. between that of the abdominal cavity and that of the surface of the body. Hart (1922) kept mice in large glass chambers at abnormal temperatures of 32° to 40°C. and at 4° to 7°C. for periods of varying numbers of days in an attempt, by experimental means, to analyze the effect of temperature on the thyroid and testes. In a description of results from one experiment he says (p. 166) that in testis (illustration 5) the spermatogenesis is completely ended and presents a picture of extreme degeneration with the majority of canals having only a single layer of sertoli cells left. That temperature is here the specific influencing factor cannot be held since changes in other glands, especially that of the thyroid, may have and very probably did have an important part in the results found in the testes. The experiments with thyroid feeding attest to this probability. Hart's experiments indicate the importance of temperature as an influencing factor though they do not show it to be the direct effect causing degeneration of the germinal epithelium. Crew (1922) has postulated high temperature as a cause of the aspermatic condition of undescended testes but offers no experimental data in support of his contentions.

SUMMARY

1. There is much disagreement among experimenters as to the effect of vasectomy upon the testes. The early workers have failed to take into account a variable factor in their experiments.

2. It was found that in all experiments where the testes resided in the scrotum following vasectomy no degeneration has taken place

3. In all experiments in which the testes were retained in the abdominal cavity with or without vasectomy, degeneration took place.

4. In those mammals where the testes are contained in a pendulous scrotum so that the results are not complicated by artificial cryptorchidism, vasectomy has not produced degeneration.

5. In birds where the position of the testes is not disturbed in performing the operation, vasectomy does not produce degeneration.

6. Testis grafts in mammals do not produce spermatozoa when placed in the abdominal cavity if their normal residence is in a scrotum. Such grafts produce spermatozoa when placed in the scrotum.

7. Vasectomy, as such, does not cause degeneration of the germinal epithelium. This has been shown in experiments of ten months' duration.

8. It is suggested that degeneration of the germinal epithelium is caused by a rise in temperature which may be either local or general in the body of the animal affected.

BIBLIOGRAPHY

ALLEN. 1919a. Anat. Record, xvi, 25.
1919b. Anat. Record, xvi, 93.
ANCEL AND BOUIN. 1903. Arch. d. Zoöl. Exper. Series 4, vol. i, 437.
BALTZER. 1912. Mittl. Zoöl. St. Neapel, 22.
BENEDICT AND SLACK. 1911. Carnegie Publication 155.
BERTHOLET. 1909. Centrabl. f. allg. Path. u. path. Anat., xx, 1062.
BRISSAUD. 1909. Arch. d. Physiol. 2nd series, vii, 769.
CASTLE AND PHILLIPS. 1911. Carnegie Inst. Wash. Publ. no. 49.
COOPER. 1827. The anatomy and surgical treatment of abdominal hernia. London, 1827.
CORDES. 1898. Virchow's Arch. path. Anat., cli, 402.
CREW. 1922. Journ. Anat. and Physiol., lvi, 98.
CURLING. 1843. A practical treatise on the diseases of the testis, and of the spermatic cord and scrotum. Philadelphia, 1843.
EVANS AND BISHOP. 1922. Journ. Metabolic Research, i, 335.
GODARD. 1856. Compt. rend. Soc. de Biol., 2d series, 111.
GODDARD. 1920. Journ. Anat., (London) liv, 173.
GOLDSCHMIDT. 1917. Endocrinol., i, 433.
GOODALE. 1916. Carnegie Inst. of Wash. Publ. no. 243.
GOSSELIN. 1853. Arch. gen. de Med., Paris 5. s. 11.
GOUBEAUX ET FOLLIN. 1855. Mem. d. la Soc. de Biol., 1855.
GRIFFITHS. 1893. Journ. Anat. and Physiol., xxvii, 483.
1894. Journ. Anat. and Physiol., xxiv, 209.
GUDERNATSCH. 1915. Anat. Record, xii, 78.
GUTHRIE. 1909. Science N.S., xxx, 724.
1910. Journ. Exper. Med., xii, 269.
1911. Science N.S., xxxiii.
GUYON. 1895. Lecons clinques sur les affections chirurgicales de la vesie prostate professées a l'Hospital, Necker, recueillies et publiees par F. D. Guiard VII, 9th. Surgical Congress, 1888.
HANES. 1911. Journ. Exper. Med., xiii, 338.
HART. 1922. Pflüger's Arch., cxcvi, 151.
HORNING AND TORREY. 1922. Anat. Record, xxv (abstract).
HOUSSAY. 1907. Arch. d. Zoöl. exper. et gen. Hist. Nat., 4s. 6, 137.
JACOBSON. 1879. Arch. f. path. Anat., lxxv.
KOSTITCH. 1921. Compt. rend. Soc. de Biol., lxxxiv, 674.
KUNTZ. 1921. Endocrinol., v, 190.
LOEB. 1918. Biol. Bull., xxxiv, 33.
LIPSCHUTZ, OTTOW and WAGNER. 1922. Proc. Royal Society, xciii, 132.
MACDOWELL AND VICARI. 1917. Proc. Nat. Acad. Sciences, iii, no. 9.
MASSAGLIA. 1920. Endocrinol., iv, 547.
MILLS. 1919. Journ. Exper. Med., xxx, 505.
MONOD ET ARTHAUD. 1887. Arch. Gen. de Med., 1887.
MOORE. 1921. Journ. Exper. Zoöl., xxxiii, 129.
1923a. Anat. Record, xxv, 142.
1923b. Science (in press).

MYERS. 1915. Anat. Record, x, 228.
PAUL. 1906. Proc. Physiol. Soc. London, Journ. Physiol., 14.
PEZARD. 1919. Compt. rend. Acad. d. Sciences, clxix, 1177.
RIDDLE. 1917. Science, N.S., xl, 19.
SAND. 1921. Journ. Physiol., xix, 305, 494, 515.
SHATTOCK AND SELIGMAN. 1904. Proc. Royal Soc. of London, lxxiii, 49.
SIMMONDS. 1921. Deutsch. Med. Wochenschr., xlvii, 665.
SMITH. 1910. Quart. Journ. Micros. Sci., liv, 577.
SPANGARO. 1904 and 1905. Rev. veneta di sci. med., xlii, 21, 80, 140, 201, 255. Lo Spermintale an 57, F. 3, Brit. Med. Journ.
STEINACH. 1921. Arch. f. Entwickmech., xlvi, 557.
STIEVE. 1921. Anat. Anz., liv, 63.
TIEDJE. 1921. Deutsch. Med. Wochenschr., xlvii, 352.
WALLACE. 1904. Trans. Path. Soc. of London, lvi, 80.
WEICHSELBAUM AND KYRLE. 1912. Sitzungsb. k. akad. Wissensch. Med. naturw. Kl., cxxi, 51.
WHEELON. 1921. Endocrinol., v, 307.
WOLBACH. 1919. Johns Hopkins Hosp Bull., xxx, 104.